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FREEZING-POINT DEPRESSION AND SPECIFIC CONDUCTIVITY OF SORGHUM TISSUE FLUIDS[1]

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INTRODUCTION

The sorghums probably are the most outstandingly drought resistant of American cultivated crops. Their ability to survive periods of moisture deficiency with little injury has long been recognized. The sorghums include extremely diverse groups within a single species. One group, the sorgos or saccharine sorghums, produces an abundance of sugar which is stored in the juice of the stalks. Grain sorghums, including kafir, milo, feterita, durra, kaoliang, and hegari, tend to produce larger quantities of seed, and more of the carbohydrates are thus diverted to starch production. Broomcorn, on the other hand, produces numerous elongated panicle branches which form the fibrous brush. The groups and varieties of sorghum show wide variations in growth habits and in disease and insect resistance. The term "sorghums" as used in this paper includes both grain sorghums and sorgos and, in some cases, broomcorn also.

The present studies were undertaken to determine differences that might exist in tissue-fluid properties of sorghum varieties under different conditions and the changes that take place in these properties. One or two varieties of corn were grown each season for comparison of their tissue-fluid properties with those of the sorghums.

EXPERIMENTAL METHODS

The experiments here reported were limited largely to determinations of the freezing-point depression and the conductivity of the expressed plant juices. In 1926, however, some determinations of total solids and "bound water" were made. Freezing-point depressions were determined by means of ordinary Beckman apparatus and a Heidenhain thermometer, and the conductivity was measured by the Wheatstone bridge at a temperature of 30° C. Total solids were measured with the Abbe refractometer, and bound water by the method of Newton and Gortner.[4]

The sorghums were grown under irrigation and received water whenever necessary to maintain growth, except in some experiments

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[2] The final draft of this manuscript was not completed until after Doctor Harris's death.

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[4] NEWTON, R., and GORTNER, R. A. A METHOD FOR ESTIMATING HYDROPHILIC COLLOID CONTENT OF EXPRESSED TISSUE FLUIDS. Bot. Gaz. 74: 442-446, illus. 1922.

in which irrigation was withheld purposely. Samples of the leaves or stalks were gathered just about sunset, stuffed into tubes, and the tubes placed in a salt-and-ice mixture for freezing. After freezing for 24 hours or more, the samples were thawed and pressed. The extracted juices were kept cold until the determinations were made. All samplings and determinations were made in duplicate and the results averaged.

When leaves from different positions on the plant were being studied, samples were taken from several plants. For other studies all or most of the leaves from a single plant were kept together. The midribs were removed from the leaf samples. The internode samples consisted of the pith with the cortical layers peeled off. They were also composites of all or most of the internodes from a single plant or of single internodes from different plants.

The experiments herein reported were conducted at the United States Field Station, Sacaton, Ariz., during the late summers of four successive years from 1926 to 1929. The results presented, while not final in all cases, are believed to be sufficiently reliable to indicate general characteristics.

RESULTS OBTAINED

LEAVES AND INTERNODES

The values for the freezing-point depression of the juices of leaves and internodes in different positions on the stalk are shown in Table 1. Data are presented for seven varieties of sorghum sampled on three dates during a period of about a month. The first sampling was made about heading time for most of the varieties, the second after the completion of blooming, and the third when the seeds were in the dough-to-ripe stage. The positions of both leaves and internodes are indicated by numbers, beginning with 1 for the upper or terminal leaf or internode and increasing downward.

The upper internode, or peduncle, protrudes from the sheath of the upper leaf and is attached at the upper node, to which the upper leaf sheath also is attached. The upper terminal leaf is the smallest in size, the second is considerably larger, while the third is the largest leaf on the stalk. Below the third leaf the size decreases gradually. The oldest leaves and internodes are nearest the base of the stalk. As the plant approaches maturity the leaves, beginning with the lower ones, gradually become dry and may break off the stalk.

The average results in Table 1 show that the freezing-point depression is greatest in the juices of the upper or terminal leaf, with a gradual decrease from the top downward. This occurred at all three periods of sampling. The concentration of the juice of all leaves was only slightly greater in the third sampling, when the seeds were nearly mature, than in the first sampling, when most of the heads were in bloom.

The freezing-point depression of the stalk juice in the various internodes was practically the same on each of the first two dates, although the concentration in all internodes had increased in the interval between the two samplings. The concentration of the juice of all internodes except the first had increased still more by the third sampling date.

By the third sampling date, when the plants were nearly mature, a variation in the juice concentration in the different internodes also was apparent, increasing from the first down to the fourth internode, with the fifth and sixth internodes about the same as the fourth.

TABLE 1.—*Freezing-point depression of the juices of leaves and internodes on three dates, Sacaton, Ariz., 1926*

Juices, date of sampling, and leaf or internode No. [a]	Freezing-point depression (° C.) of—							
	Dwarf Yellow milo	Dawn kafir	Reed kafir	Dwarf hegari	Standard feterita	Black Amber sorgo	Kansas Orange sorgo	Average
Leaf juices:								
July 28 or 30, from leaf No.—								
1	1.04	0.96	0.79	1.31	1.10	0.93	0.94	1.01
2	.99	.88	.92	1.06	1.03	.95	.97	.97
3	.98	.86	.93	.93	.98	.89	1.06	.95
4	[b] 1.00	.83	.94	.87	.92	.83	1.07	.92
5	1.03	.81	.95	.82	.86	.82	.98	.90
6	.92	.78	.91	.82	.86	.81	.93	.86
7				.94				
Aug. 5 or 9, from leaf No.—								
1		.92		.91	1.08	.86		[c] .94
2	1.04	.85		.92	1.06	.88		[c] .93
3	1.05	.84		.80	.97	.81		[c] .86
4	1.02	.82		.84	.93	.80		[c] .85
5	.98	.81		.77	.90	.78		[c] .82
6	.97	.78		.81	.85	.76		[c] .80
7	.98	.77		.73	.82	.76		[c] .77
8	.99	.79			.77			
9	.96	.81			.78			
Aug. 28 to Sept. 1, from leaf No.—								
1	.94	1.00	1.04	1.07	1.06	1.17	1.13	1.06
2	.95	.91	.99	1.00	1.02	1.15	1.16	1.03
3	.92	.87	.95	.92	.94	1.08	1.17	.98
4	.92	.88	.92	.90	.94	1.03	1.18	.97
5	.90	.86	.88	.88	.90	.98	1.15	.94
6	.91	.81	.82	.87	.90	.96	1.13	.91
7		.81	.77		.92	.90	1.11	
8							1.09	
9							1.05	
Internode juices:								
July 28 or 29, from internode No.—								
1	.95	1.03		[d] 1.11	1.24	1.14		1.09
2	1.04	1.01		[d] 1.20	1.28	1.08		1.12
3	.96	.98		[d] 1.23	1.28	1.05		1.10
4	1.14	.99		[d] 1.24	1.28	1.08		1.15
5	.99	.91		[d] 1.25	1.28	1.03		1.09
6	1.11	.88		[d] 1.27	1.28	1.04		1.12
7				[d] 1.29				
8				[d] 1.24				
9				[d] 1.30				
Aug. 5 or 9, internode No.—								
1	1.32	1.38			1.37	1.33		1.35
2	1.19	1.33			1.36	1.26		1.29
3	1.29	1.35			1.34	1.21		1.30
4	1.27	1.38			1.22	1.26		1.28
5	1.22	1.34			1.28	1.16		1.25
6	1.27	1.29			1.29	1.28		1.28
7	1.21	1.27			1.26	1.11		1.21
8	1.24	1.27			1.35	1.16		1.26
9	1.20	1.27			1.45	1.20		1.28
Aug. 28 to Sept. 1, from internode No.—								
1	1.30	1.39	1.30	1.29	1.45	1.35	1.57	[e] 1.36
2	1.50	1.65	1.46	1.61	1.59	1.47	1.77	[e] 1.56
3	1.51	1.69	1.47	1.64	1.73	1.56	1.84	[e] 1.63
4	1.54	1.79	1.52	1.79	1.85	1.70	1.91	[e] 1.73
5	1.55	1.62	1.49	1.74	1.88	1.67	1.92	[e] 1.69
6	1.45	1.60	1.44	1.87	1.89	1.65	1.86	[e] 1.69
7			1.44		1.86	1.63	1.86	
8							1.77	
9							1.70	

[a] Beginning with terminal leaf or internode.
[b] Interpolated from leaves 3 and 5.
[c] Not including Dwarf Yellow milo.
[d] Aug. 5, Dwarf hegari was late.
[e] Not including Reed kafir and Kansas Orange sorgo.

The average specific conductivity of the juices from leaves in different positions, shown in Table 2, was nearly the same at all three samplings, although the conductivity of the lowest leaves was slightly less than the others. The average conductivity of the juices from the third sampling of leaves was slightly higher than that of the first sampling.

TABLE 2.—*Specific conductivity of the juices of leaves and internodes at three dates, Sacaton, Ariz., 1926*

Juices, date of sampling, and leaf or internode No.[a]	Specific conductivity (reciprocal ohms) of—							
	Dwarf Yellow milo	Dawn kafir	Reed kafir	Dwarf hegari	Standard feterita	Black Amber sorgo	Kansas Orange sorgo	Average
Leaf juices:								
July 28 or 30, from leaf No.—								
1	0.0107	0.0164	0.0120	0.0207	0.0122	0.0120		[b] 0.0147
2	.0130	.0153	.0150	.0182	.0126	.0131	0.147	[b] .0148
3	.0139	.0161	.0161	.0169	.0127	.0128	.0144	[b] .0149
4		.0147	.0170	.0165	.0126	.0119	.0148	[b] .0145
5	.0150	.0150	.0176	.0161	.0125	.0115	.0159	[b] .0145
6	.0139	.0130	.0172	.0148	.0128	.0111	.0166	[b] .0138
August 5 or 9, from leaf No.—								
1		.0156		.0159	.0140			[c] .0155
2	.0135	.0151		.0161	.0148	.0159		[c] .0157
3	.0162	.0157		.0159	.0146	.0165		[c] .0153
4	.0166	.0114		.0178	.0157	.0161		[c] .0153
5	.0156	.0155		.0155	.0146	.0155		[c] .0150
6	.0154	.0141		.0158	.0149	.0152		[c] .0137
7	.0140	.0144		.0144	.0147	.0114		
8	.0114	.0107			.0153			
9		.0132			.0141			
August 28 to September 1, from leaf No.—								
1		.0162	.0180	.0179	.0153	.0166	.0159	[b] .0168
2	.0156	.0169	.0187	.0182	.0159	.0171	.0183	[b] .0174
3	.0161	.0167	.0190	.0166	.0161	.0179	.0197	[b] .0173
4	.0174	.0172	.0182	.0170	.0161	.0170	.0200	[b] .0171
5	.0164	.0170	.0177	.0166	.0151	.0164	.0181	[b] .0166
6	.0171	.0161	.0168	.0152	.0151	.0155	.0201	[b] .0157
7		.0161	.0156			.0140	.0184	
8							.0202	
9							.0191	
Internode juices:								
July 28 or 30, from internode No.—								
1	.0066	.0092		[d] .0077	.0162	.0085		[e] .0080
2	.0101	.0083		[d] .0113	.0191	.0068		[e] .0091
3	.0085	.0101		[d] .0131	.0172	.0061		[e] .0095
4	.0141	.0099		[d] .0135	.0172	.0072		[e] .0112
5	.0151	.0104		[d] .0138	.0148	.0062		[e] .0114
6	.0176	.0124		[d] .0135	.0153	.0087		[e] .0131
7				[d] .0139				
8				[d] .0164				
9				[d] .0182				
August 5 or 9, from internode No.—								
1	.0109	.0132			.0173	.0098		.0128
2	.0135	.0092			.0161	.0070		.0115
3	.0198	.0119			.0183	.0080		.0145
4	.0249	.0128			.0138	.0066		.0145
5	.0243	.0123			.0139	.0075		.0145
6	.0225	.0126			.0144	.0065		.0140
7	.0247	.0155			.0141	.0124		.0167
8	.0240	.0198			.0156	.0126		.0180
9	.0237	.0191			.0171	.0141		.0185
August 28 to September 1, from internode No.—								
1	.0248	.0174	.0171	.0245		.0199	.0127	[e] .0217
2	.0247	.0141	.0162	.0263	.0282	.0130	.0083	[e] .0195
3	.0280	.0151	.0193	.0228	.0187	.0107	.0092	[e] .0192
4	.0317	.0144	.0210	.0195	.0163	.0091	.0089	[e] .0187
5	.0321	.0140	.0192	.0202	.0151	.0090	.0087	[e] .0188
6	.0290	.0136	.0182	.0180	.0151	.0096	.0075	[e] .0176
7		.0140	.0167		.0149	.0094	.0079	
8							.0086	
9							.0078	

[a] Beginning with terminal leaf or internode.
[b] Not including Dwarf Yellow milo and Kansas Orange sorgo.
[c] Not including Dwarf Yellow milo.
[d] Aug. 5, Dwarf hegari was late.
[e] Average of Dwarf Yellow milo, Dawn kafir, Dwarf hegari, and Black Amber sorgo.

The juices of the various internodes show decided differences in conductivity. In the first sampling the average conductivity increased rapidly from the upper internode downward, and a similar but somewhat irregular tendency prevailed at the second sampling. This situation was reversed, however, in the last sampling, as the upper internodes showed the highest conductivity. The average conductivity of the juice of all internodes was much higher in the final sampling than in the first sampling.

It is evident from the above results that food materials do not accumulate in the leaf juices of sorghum plants at maturity. Collier [5] obtained a decrease in sugars in sorgo leaf juices, an increase in "solids not sugar," and a slight increase in the specific gravity of leaf juices between blooming and maturity.

The internodes of sorghums serve as storage reservoirs for sugars. As the plant approaches maturity after the flowering period, sugars increase in the internodes, the greatest accumulation being in the fourth and lower internodes. The salts, on the other hand, accumulate to the greatest extent in the upper internodes with a gradual decrease downward in the stalk. The higher conductivity in the upper internodes probably is partly due to earlier drying in this region as the plants approach maturity.

The concentration gradient in sorghum stalks, as measured by the freezing-point depression, did not correspond to the falling gradient from the top downward established for cornstalks by Hurd-Karrer [6] through specific-gravity measurements of internode juices. The freezing-point depression of the leaf juices at all stages and the conductivity of the sorghum internode juices at maturity, however, showed the same tendency toward a decrease from the top down as reported for corn.

Collier [7] showed that the lower and middle portions of stalks of sorgo, corn, and sugarcane contained more juice and sugars than the top portions. The juices in the top portion of sugarcane stalks contained more salts or ash than those in the middle and butt. The juice from the tops of sorghum stalks contained more "solids not sugar" (chiefly ash and inorganic acids) than that from the middles and butts. The specific gravity and the total-solids content of the juices were nearly constant in different portions of the stalk of corn and sorghums. In sugarcane, however, the juices of the tops were lower than those of the middles and butts in both specific gravity and solids. Collier's results indicated that salts accumulate mostly in the upper portion of the stalk, and sugars in the middle and lower portion.

CROWNS AND ROOTS

Data from a few determinations on the juices of the crowns and roots of sorghum and corn are shown in Table 3. The crown samples consisted of the basal portion of the stalks below the surface of the soil with the roots and the cortical tissues removed. The root samples consisted of both large and small roots cut from the crown and wiped free from dirt with a dry cloth. In all the experiments the Kansas Orange sorgo and Sacaton June corn were grown in adjacent

[5] COLLIER, P. SORGHUM; ITS CULTURE AND MANUFACTURE ECONOMICALLY CONSIDERED AS A SOURCE OF SUGAR, SYRUP, AND FODDER. 570 p., illus. Cincinnati. 1884.

[6] HURD-KARRER, A. M. A CONCENTRATION GRADIENT IN CORNSTALKS. Jour. Gen. Physiol. 9: 341-343. 1926.

[7] COLLIER, P. Op. cit. (See footnote 5.)

rows. In 1928 and 1929 these two varieties were flanked by rows of Red kafir and Reid corn, from which samples were obtained. The Dwarf Yellow milo and Standard feterita mature rather early; Kansas Orange sorgo, Red kafir, and Sacaton June corn mature late but all three at about the same time.

The data in Table 3 indicate that the osmotic concentration of the juices of the crowns, as measured by the freezing-point depression, usually is higher in sorghum than in corn. The juice of the roots of sorghums has a much higher osmotic concentration than that of corn. Evidently corn roots do not carry nearly so much material in solution as do sorghum roots.

TABLE 3.—*Freezing-point depression and specific conductivity on different dates of crown and root juices of four varieties of sorghum and two varieties of corn*

Plant part and date of sampling	Freezing-point depression (° C.) of—						Specific conductivity (reciprocal ohms) of—					
	Dwarf Yellow milo	Red kafir	Standard feterita	Kansas Orange sorgo	Sacaton June corn	Reid corn	Dwarf Yellow milo	Red kafir	Standard feterita	Kansas Orange sorgo	Sacaton June corn	Reid corn
Crown juices:												
Aug. 15, 1927				1.15	0.96					0.0183	0.0268	
Aug. 27, 1927				1.82	1.51					.0273	.0451	
Aug. 24, 1928	1.21	0.92	1.18	1.05	.86	0.91	0.0127	0.0173	0.0157	.0121	.0224	0.0225
Aug. 30, 1928	1.01	1.01		1.05	.86	.92	.0213	.0272		.0183	.0373	.0301
Sept. 4, 1928 [a]		.89		.96	.94			.0190		.0128	.0267	
Aug. 22, 1929		1.08			1.06	1.13		.0187			.0292	.0316
Aug. 27, 1929		1.07			.89	.99		.0171			.0265	
Root juices:												
Sept. 2, 1926				1.56	.76					.0178	.0215	
Aug. 10, 1927				1.28	1.06							
Aug. 27, 1927				1.83	1.49					.0266	.0349	
Aug. 30, 1928	1.12	1.11		1.21	.76	.83	.0251	.0206		.0238	.0309	.0311
Sept. 4, 1928 [b]		1.05		1.20	.83			.0174		.0182	.0230	
Aug. 22, 1929		1.28			.88	.90		.0201			.0252	.0240
Aug. 27, 1929		1.23			.80	.84		.0195			.0218	.0226

[a] Juice from less mature plants than samples taken Aug. 24 and 30, 1928.
[b] Juice from less mature plants than samples taken Aug. 30, 1928.

Conductivity determinations, on the other hand, show that the crowns and roots of corn contain considerably greater quantities of electrolytes than do sorghum crowns and roots. The higher freezing-point depression in sorghum crowns and roots is due, therefore, to nonelectrolytes, probably sugars. Conrad [8] has shown that considerable quantities of sugar are in sorghum roots. Wilson and Wilson [9] reported that sorghum roots contain nearly three times as much soluble organic matter as corn roots.

The higher osmotic concentration of the juices of crowns and roots may account in part for the fact that sorghum is better able than corn to resist drought. This high concentration would help to prevent tissue desiccation and might aid in removing water from a relatively dry soil.

The crowns and roots of sorghum plants often remain alive during extended periods of drought and send up tillers later, when moisture becomes available. Frequently a fair crop of grain sorghum is produced from the tillers alone.

[8] CONRAD, J. P. FERTILIZER AND LEGUME EXPERIMENTS FOLLOWING SORGHUMS. Jour. Amer. Soc. Agron. 20: 1211-1234, illus. 1928.
[9] WILSON, B. D., and WILSON, J. K. RELATION OF SORGHUM ROOTS TO CERTAIN BIOLOGICAL PROCESSES. Jour. Amer. Soc. Agron. 20: 747-754. 1928.

TILLERS AND BRANCHES

Tillers develop from buds on the crown nodes of sorghum plants. Branches or offshoots, when present, develop on the sorghum stalk after the head is mature, from buds in the axils of the leaf sheaths. Table 4 shows the freezing-point depression and conductivity of the juices of leaves and internodes of main stalks, tillers, and branches on the same sorghum plants. Leaves from main stalks and from tillers of Dwarf Yellow milo and Dawn kafir show nearly the same freezing-point depression and conductivity, determined as the average of six plants. The internodes, on the other hand, show striking differences, the juice of main-stalk internodes being considerably greater in both freezing-point depression and conductivity than the juice from tiller internodes.

TABLE 4.—*Freezing-point depression and conductivity of the leaf and internode juices and moisture content of main stalks, tillers, and branches of sorghum varieties*

Variety and plant part	Freezing-point depression (° C.) of—		Specific conductivity (reciprocal ohms) of—		Moisture content of internodes
	Leaves	Internodes	Leaves	Internodes	
					Per cent
Dwarf Yellow milo:					
Main stalks	1.01	1.37	0.0181	0.0149	.....
Tillers	1.03	.96	.0189	.0120	.....
Dawn kafir:					
Main stalks	.88	1.60	.0159	.0120	.....
Tillers	.81	1.13	.0157	.0091	.....
Standard feterita:					
Upper main stalks	.89	1.56	.0131	.0347	64.4
Lower main stalks	.87	1.61	.0169	.0257	79.1
Branches	.90	1.31	.0137	.0163	76.4
White durra:					
Upper main stalks	1.19	1.41	.0155	.0327	63.3
Lower main stalks	1.09	1.41	.0141	.0200	83.2
Branches	1.12	1.06	.0140	.0113	85.3
Black Amber sorgo:					
Upper main stalks	1.07	1.30	.0174	.0142	84.5
Lower main stalks	1.02	1.41	.0199	.0101	83.4
Branches	.89	1.13	.0113	.0063	85.9

Similar results were obtained by comparing the main stalks and branches of three sorghum varieties. Both tillers and branches are essentially young stalks that have not yet accumulated large quantities of stored materials. The leaf juices of the branches were very similar to those of the main stalks except in Black Amber sorgo. The internode juices of the branches, however, show considerably less freezing-point depression and conductivity than the juices from either the upper or lower internodes on the main stalks. The upper internodes consisted of the peduncle and second internode, both of which are above the base of the topmost branch.

Differences in moisture content of the branches and of the upper and lower main-stalk internodes, as shown in Table 4, may account in part for the differences in conductivity between the upper and lower main stalks in Standard feterita and White durra, but not in Black Amber sorgo.

VARIETAL DIFFERENCES

Determinations of the freezing-point depression and conductivity of the juices of different sorghum varieties were made at various times during four seasons. Often the tests were limited to two or

three varieties sampled on a single day. No two varieties were at exactly the same stage of maturity at a given time, and all varieties were changing rapidly during growth and maturation. Differences in soil and soil moisture and in the condition of the plants may have affected the results considerably. Table 5 shows the measurements of average and extreme freezing-point depression and conductivity of the juices of seven varieties of sorghum and one variety of corn. The data include measurements of composite samples of the juice of all or most of the leaves or internodes of a plant and averages of the determinations of individual leaves or internodes.

The data in Table 5 indicate a wide variability in the character of plant juices sampled under different conditions of season, maturity, and moisture. The average results for each variety indicate that in both freezing-point depression and conductivity only small differences exist between the leaf juices of different varieties.

The leaf juices of Sacaton June corn were higher than those of any sorghum in both characters. Larger varietal differences occurred in the stalks than in the leaves, the two sorgos and Standard feterita showing the greatest freezing-point depression and Sacaton June corn the least. In conductivity of stalk juices, Standard feterita was the highest, followed by Dwarf Yellow milo. The two sorgos showed the lowest conductivity. Of the sorghum varieties, feterita showed the highest conductivity of internode juices and the lowest conductivity of leaf juices, whereas Kansas Orange sorgo showed the lowest conductivity in the stalks and the highest in the leaves. These relationships did not hold for all varieties.

TABLE 5.—*Maximum, minimum, and average freezing-point depression and conductivity of the leaf and internode juices of seven varieties of sorghum and one variety of corn at Sacaton, Ariz., during four seasons*

FREEZING-POINT DEPRESSION

Variety	Leaves				Internodes			
	Samples	Maximum	Minimum	Average	Samples	Maximum	Minimum	Average
	Number	°C.	°C.	°C.	*Number*	°C.	°C	°C
Dwarf Yellow milo	23	1.22	0.85	1.00	20	1.55	0.94	1.25
Dawn kafir	21	1.16	.79	.91	18	1.77	.97	1.41
Reed kafir	17	1.21	.79	.95	13	1.65	.98	1.28
Dwarf hegari	18	1.23	.78	1.01	14	1.92	.85	1.37
Standard feterita	17	1.12	.77	.90	14	1.76	1.23	1.50
Black Amber sorgo	17	1.17	.66	.92	13	1.58	1.23	1.35
Kansas Orange sorgo	19	1.35	.86	1.11	15	2.10	1.01	1.64
Sacaton June corn	20	1.81	.89	1.16	15	1.76	.79	1.19

SPECIFIC CONDUCTIVITY

	Number	*Reciprocal ohms*	*Reciprocal ohms*	*Reciprocal ohms*	*Number*	*Reciprocal ohms*	*Reciprocal ohms*	*Reciprocal ohms*
Dwarf Yellow milo	23	0.0206	0.0133	0.0169	20	0.0327	0.0116	0.0193
Dawn kafir	21	.0234	.0140	.0167	18	.0162	.0100	.0129
Reed kafir	17	.0201	.0152	.0176	13	.0247	.0123	.0170
Dwarf hegari	18	.0229	.0144	.0168	14	.0219	.0091	.0129
Standard feterita	17	.0209	.0121	.0150	14	.0321	.0141	.0222
Black Amber sorgo	18	.0180	.0113	.0154	14	.0156	.0072	.0111
Kansas Orange sorgo	19	.0212	.0145	.0178	15	.0117	.0069	.0084
Sacaton June corn	20	.0330	.0156	.0215	14	.0216	.0093	.0143

TABLE 6.—*Freezing-point depression and conductivity of tissue fluids, and moisture content of internodes of 16 varieties of sorghum and 2 varieties of corn at Sacaton, Ariz., 1928 and 1929*

FREEZING-POINT DEPRESSION (°C.)

Group and variety	Leaf juices sampled						Internode juices sampled						Average moisture content of internodes [a]
	1928		1929				1928		1929				
	Aug. 24 or 25	Aug. 31 or Sept. 3	Aug. 20 or 21	Aug. 24 or 26	Aug. 30 or 31	Average	Aug. 24 or 25	Aug. 31 or Sept. 3	Aug. 20 or 21	Aug. 24 or 26	Aug. 30 or 31	Average	
													Per cent
Milo:													
Dwarf Yellow	1.16	1.02	0.90	1.03	0.86	0.99	1.30	1.52	1.27	1.41	1.21	1.34	(a)
Feterita:													
Standard	1.01	.92	.78	.83	.77	.86	1.45	1.47	1.44	1.53	1.38	1.45	.....
Durra:													
White	1.04	.....	.95	.90	.99	.....	1.10	.....	1.65	1.53	1.60	.....	.....
Kafir:													
Dawn	1.16	.86	.80	.89	.79	.90	1.77	1.68	1.48	1.71	1.20	1.57	.....
Reed	1.21	.97	.79	.92	.82	.94	1.27	1.46	1.25	1.34	1.16	1.30	.....
Sunrise	1.34	.97	.95	.97	.92	1.03	1.26	1.57	1.40	1.58	1.42	1.45	.....
Texas Blackhull	1.12	.90	.....	.....	.....	.....	1.24	1.42	.....	.....	.....	.....	.....
Red	1.15	1.12	.95	.91	.93	1.01	1.24	1.30	1.34	1.31	1.49	1.34	.....
Hegari:													
Dwarf	.97	.91	.85	1.04	.93	.94	1.30	1.21	1.13	1.27	1.64	1.31	.....
Kaoliang:													
Manchu Brown	.....	.....	1.00	.86	1.01	.....	.....	.....	1.53	1.44	1.61	.....	.....
Sorgo:													
Black Amber	1.17	.97	.89	.93	.86	.96	1.44	1.42	1.24	1.11	1.21	1.28	.....
Kansas Orange	1.15	1.12	.93	1.05	.89	1.03	1.43	1.80	1.40	1.58	1.64	1.57	.....
Leoti Red	1.30	1.00	1.07	.94	.98	1.06	1.43	1.54	1.52	1.58	1.51	1.52	.....
Freed	1.09	.99	.92	.....	.....	.....	1.37	1.42	1.37	.....	.....	.....	.....
Atlas	.....	.....	1.02	.92	.98	.....	.....	.....	1.48	1.63	1.50	.....	.....
Broomcorn:													
White Italian	.....	.....	1.28	1.01	1.10	.....	.....	.....	1.50	1.56	1.47	.....	.....
Corn:													
Sacaton June	1.29	1.08	.92	1.13	1.16	1.12	1.17	.99	1.07	1.02	1.13	1.08	.....
Reid	1.51	1.00	1.10	1.20	1.02	1.17	1.26	.89	1.04	1.25	1.36	1.16	.....

CONDUCTIVITY (RECIPROCAL OHMS)

Milo:													
Dwarf Yellow	0.0178	0.0172	0.0167	0.0162	0.0163	0.0168	0.0131	0.0142	0.0128	0.0144	0.0162	0.0141	82.9
Feterita:													
Standard	.0140	.0153	.0137	.0160	.0162	.0150	.0141	.0204	.0190	.0213	.0238	.0197	81.2
Durra:													
White	.0157	.....	.0158	.0155	.0155	.....	.0161	.....	.0208	.0208	.0216	.....	77.3
Kafir:													
Dawn	.0169	.0184	.0161	.0163	.0171	.0170	.0101	.0126	.0108	.0103	.0156	.0119	82.0
Reed	.0189	.0188	.0168	.0179	.0188	.0182	.0123	.0146	.0138	.0136	.0180	.0145	84.0
Sunrise	.0161	.0184	.0174	.0173	.0174	.0173	.0101	.0117	.0158	.0168	.0161	.0141	82.3
Texas Blackhull	.0163	.0175	.....	.....	.....	.....	.0157	.0189	.....	.....	.....	.....	81.8
Red	.0170	.0181	.0170	.0162	.0158	.0168	.0129	.0169	.0144	.0168	.0161	.0154	84.7
Hegari:													
Dwarf	.0155	.0229	.0151	.0154	.0172	.0172	.0116	.0122	.0104	.0120	.0142	.0121	83.3
Kaoliang:													
Manchu Brown	.....	.....	.0197	.0170	.0179	.....	.....	.....	.0229	.0261	.0277	.....	77.1
Sorgo:													
Black Amber	.0142	.0180	.0165	.0176	.0177	.0168	.0077	.0116	.0098	.0131	.0143	.0113	85.6
Kansas Orange	.0153	.0145	.0157	.0177	.0175	.0161	.0069	.0074	.0085	.0112	.0082	.0084	86.2
Leoti Red	.0171	.0165	.0187	.0180	.0171	.0175	.0088	.0118	.0122	.0123	.0126	.0115	82.8
Freed	.0175	.0173	.0173	.....	.....	.....	.0124	.0176	.0151	.....	.....	.....	82.3
Atlas	.....	.....	.0174	.0163	.0162	.....	.....	.....	.0133	.0142	.0147	.....	85.2
Broomcorn:													
White Italian	.....	.....	.0223	.0177	.0187	.....	.....	.....	.0266	.0272	.0291	.....	78.5
Corn:													
Sacaton June	.0190	.0225	.0168	.0179	.0203	.0196	.0122	.0122	.0116	.0201	.0216	.0155	81.7
Reid	.0221	.0236	.0197	.0196	.0190	.0208	.0096	.0090	.0180	.0181	.0122	.0134	83.6

[a] Average of 4 determinations, 2 each year.

Determinations made on 16 sorghum and 2 corn varieties in 1928 and 1929 are shown in Table 6. These samples were obtained under average and fairly comparable conditions. The most obvious distinction between the juices of sorghum varieties is in the conductivity of the juices of the internodes. In general there seems to be a tendency for varieties within the different groups of sorghums to show similar conductivities. Feterita, durra, broomcorn, and kaoliang, which have dry or pithy stalks, have a high conductivity of the internode juices. Collier [10] showed durra stalk juices to be considerably higher than sorgos in "solids" (mostly salts and organic acids). The sorgos have a comparatively low conductivity of stalk juices.

The kafirs and the milo seem to be intermediate in conductivity and in stalk juiciness between the very dry-stalked sorghums and the sorgos. Dwarf hegari is similar to kafir in the juiciness of the stalk, in the appearance of the heads and seeds, and in tissue-fluid properties.

The leaf juices of broomcorn and corn have a greater freezing-point depression and conductivity than was obtained in the grain sorghums and sorgos. All sorghums exceed corn in the freezing-point depression of the stalk juices.

RELATION TO MOISTURE CONTENT

The stalks of feterita, durra, broomcorn, and kaoliang are rather dry, whereas kafir stalks are juicy and sorgo stalks extremely juicy. This indicates a general tendency for juiciness to be inversely proportional to the conductivity of the juice. Some moisture-content determinations were made in 1928 and 1929 to test this relationship. Portions of the peeled internodes prepared for the juice extraction were saved for the moisture determinations, and the average moisture content of these is shown in Table 6. The moisture content of the internodes of 14 sorghum varieties in 1928 ranged from 78 to 87 per cent, and in 1929 the average moisture content of duplicate collections of 13 varieties varied from 77 to 89 per cent.

There was some relationship between the moisture content of the stalks and the conductivity of the tissue fluids in sorghum stalks, a negative but not significant correlation coefficient of -0.28 ± 0.12 being obtained in 1928 and a larger and quite significant coefficient of -0.61 ± 0.08 in 1929.

There was no definite relation between the moisture content of stalks and the freezing-point depression of the juices, because sorgo stalks that are very juicy also contain considerable quantities of dissolved sugar.

SEASONAL CHANGES

No determinations were made on young sorghum plants, because of the difficulty of getting samples of comparable leaves and internodes before the plants reach the heading stage. Changes that took place between heading and ripening, however, are shown by the data for the three periodic samplings of 1926 (Tables 1 and 2) and by the differences between main stalks and tillers (Table 4). There was little change in either freezing-point depression or conductivity of the leaf juices as the plants approached maturity. In the internodes, how-

[10] COLLIER, P. Op. cit. (See footnote 5.)

ever, there was a marked accumulation of soluble materials in the juices as the stalks neared maturity. Both the freezing-point depression and the conductivity may increase 50 to 75 per cent within a month. Collier [11] showed average changes between heading and ripening as follows: Sucrose increased from 4.23 to 11.75 per cent of the juice, glucose decreased from 3.89 to 2.09 per cent, "solids not sugars" increased from 2.36 to 2.82 per cent, while the specific gravity increased from 1.043 at the heading stage to 1.068 at maturity.

EFFECT OF MOISTURE SUPPLY

In order to determine the changes that occur when the plants are subjected to the stress of drought, a plot on which seven varieties of sorghum and one of corn were growing was left unirrigated during a dry period in 1927. Another plot, similarly prepared, was kept supplied with irrigation water as needed. After the leaves on the plants in the delayed-irrigation plot had begun to show severe firing, samples were taken and the land was irrigated the following day. On the day following this irrigation, samples again were taken. Table 7 shows the data obtained before and after the delayed irrigation, together with comparable data from samples from the plot receiving normal irrigation.

TABLE 7.—*Freezing-point depression and specific conductivity of the leaf and internode juices of seven varieties of sorghum and one variety of corn from a dry plot before and after a delayed irrigation and from a plot normally irrigated*

Variety	Freezing-point depression (° C.) under indicated treatment						Specific conductivity (reciprocal ohms) under indicated treatment					
	Leaf juices			Internode juices			Leaf juices			Internode juices		
	Delayed irrigation		Normal irrigation	Delayed irrigation		Normal irrigation	Delayed irrigation		Normal irrigation	Delayed irrigation		Normal irrigation
	Before	After		Before	After		Before	After		Before	After	
Dwarf Yellow milo	0.89	0.92	1.06	1.03	1.30	1.19	0.0159	0.0175	0.0189	0.0275	0.0281	0.0286
Dawn kafir	.96	.92	1.08	1.45	1.31	1.39	.0195	.0184	.0168	.0120	.0153	.0132
Reed kafir	.93	.96	.92	1.07	1.17	.98	.0183	.0201	.0168	.0247	.0198	.0220
Dwarf hegari	1.06	1.20	1.23	.85	1.92	1.54	.0175	.0204	.0156	.0129	.0116	.0157
Standard feterita	.95	.....	1.03	1.70	.....	1.76	.0121	.....	.0209	.0214	.....	.0308
Black Amber sorgo	1.07	.....	.98	1.39	.....	1.42	.0171	.....	.0129	.0137	.....	.0156
Kansas Orange sorgo	1.27	1.29	1.35	2.10	1.91	1.99	.0174	.0212	.0184	.0086	.0091	.0071
Average of 7 varieties	1.02	.....	1.09	1.37	.....	1.47	.0168	.....	.0172	.0173	.....	.0190
Average of 5 varieties	1.02	1.06	1.13	1.30	1.52	1.42	.0177	.0195	.0173	.0174	.0168	.0173
Sacaton June corn	1.81	1.46	1.22	1.76	1.41	1.21	.0330	.0250	.0234	.0161	.0146	.0132

The average results for the seven sorghum varieties show that the freezing-point depression and conductivity of the juices of both leaves and internodes were slightly greater in the plants receiving normal irrigation than in plants from which water was withheld. This shows that the osmotic concentration of sorghum juices is not increased by water deficiency. The juices of the leaves and internodes of Sacaton June corn showed a higher concentration in all cases in the plants

[11] COLLIER, P. Op. cit. (See footnote 5.)

from which water was withheld than in the normally irrigated plants. The corn plants were severely wilted, but the sorghum plants showed only slight wilting; this probably explains the differences found in the tissue fluids of the two species.

Only five varieties of sorghums were sampled after the delayed irrigation, because the Standard feterita and Black Amber sorgo were mature at this time. Increases in the average freezing-point depression of both leaves and internodes of the sorghums and in the conductivity of the leaves were obtained after the delayed irrigation. This apparently indicated a resumption of nutrient absorption and of photosynthesis by the sorghums. The corn-plant juices, on the other hand, all showed definite decreases in freezing-point depression and conductivity after the delayed irrigation, indicating that the absorption of water was the most important change taking place.

Collier[12] found a difference of only 0.003 in the average specific gravity of the juice of 36 varieties of sorgo taken before and after a heavy rainfall following a long drought.

TOTAL SOLIDS AND BOUND WATER

The percentage of total solids in the juices, as measured by the sugar scale of the Abbe refractometer, was determined in a considerable number of samples of leaf and internode juices in 1926. The total-solids measurements corresponded in general with the freezing-point depression determinations but were determined less accurately. The total solids in the leaf juices of sorghums varied from 6.5 to 14.3 per cent and those in Sacaton June corn from 6.2 to 13.7 per cent. The total solids of the internode juices varied from 7.2 to 19.4 per cent in sorghums and from 10.1 to 11.9 per cent in Sacaton June corn.

The method used showed no indication of bound water in the leaf juices that could not be accounted for by experimental error. Internode juices apparently contained no bound water before the plants were nearly mature. When nearing maturity, however, there was some indication of bound water in the internode juices of sorghum but none in corn. Irregularities in the data preclude definite conclusions, but the presence of bound water, even in the stalks, appears doubtful.

CONCLUSIONS AND SUMMARY

The freezing-point depression is greatest in the juices of the terminal leaf of sorghums, with a progressive decrease in the leaf juices from the top of the plant downward.

The freezing-point depression of the juices of different internodes is about the same in the flowering stage but increases from the top down to the fourth internode when the plant approaches maturity.

The conductivity of the juices of leaves in different positions is nearly the same.

The juices of the upper internodes have the lowest conductivity in the flowering stage and the highest in the milk or dough stage.

The crowns and roots of sorghums have a higher osmotic concentration than those of corn, but a lower conductivity. The difference in osmotic concentration may partly account for the greater drought resistance of sorghums.

[12] COLLIER, P. Op. cit. (See footnote 5.)

The juices of tillers and branches of sorghums have less freezing-point depression and a lower conductivity than the older main stalks.

The leaf juices of sorghums show only small varietal differences. The conductivities of the internode juices show considerable varietal differences and are high in feterita and durra, low in sorgos, and intermediate in kafirs.

Sorghum-leaf juices (except broomcorn) have less freezing-point depression and a lower conductivity than corn.

Sorghum-stalk juices usually have a greater freezing-point depression than those of corn. The average conductivity of sorghum-stalk juices is about the same as that of corn.

The differences in conductivity of stalk juices in sorghums can be accounted for to a considerable extent by differences in the juiciness of the stalks.

There is little change in either freezing-point depression or conductivity of leaf juices as the plants approach maturity. The internode juices may increase 50 to 75 per cent in freezing-point depression and specific conductivity between heading and ripening.

Water deficiency does not cause an increase in the freezing-point depression or conductivity of the leaf and stalk juices in sorghum, but may in corn if the plants wilt.

There was no definite indication of bound water in the juices of sorghums.

GERMINATION LOSS OF CONIFEROUS SEEDS DUE TO PARASITES[1]

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INTRODUCTION

Literature on damping off (*2, 3, 8, 18, 22, 30, 34*)[2] contains scattered references to germination loss caused by damping-off fungi to various hosts, but there are comparatively few references to that of conifers. Spaulding (*32, p. 74*), in discussing the damping off of coniferous seedlings, reported that "while damping-off may kill the germinating seedlings before they push through the soil, it usually attacks them a few days after the seedlings have come up." Hartley (*10, 11, 12*) stated that *Pythium debaryanum*, *Corticium vagum* (*Rhizoctonia solani*), and *Fusarium* spp. all caused germination loss in coniferous-seed beds. The best data concerning germination loss under nursery conditions are furnished by Hartley's analyses (*11*) by several methods of the results with several species of pine in various soil-disinfection tests. He found evidence that some such loss nearly always occurred, that in some cases the loss before the seedlings emerged from the soil was apparently considerably greater than the damping off that took place later, and that in extreme cases the estimated germination loss equaled approximately four-fifths of the total number of viable seeds. These disinfectant tests gave little indication as to which fungi were responsible for the loss. Inoculation experiments in which inoculum had been added to the soil instead of being placed in direct contact with the seeds or seedlings also did not show whether certain fungi failed to cause germination loss because of lack of parasitic ability or because of inability to grow well in the soils used. None of the previous tests furnished data as to whether the fungi not only caused a very early rotting of the radicles but also decayed viable unruptured seeds. This information is important in determining whether seeds planted in the fall and those that normally do not germinate until the second or third year after planting will be attacked by damping-off fungi while dormant. In order to secure this information coniferous seeds were directly inoculated with damping-off fungi. Results secured by the direct inoculation of stems, taproots, and older roots with the same fungi can be found elsewhere (*27, 28, 29*).

METHODS OF INOCULATION

A few small preliminary trials (*26*) in which seeds were sown broadcast on the surface of agar of Petri-dish cultures of the tested fungi demonstrated this method to be very unsatisfactory. Thereupon the agar was removed from two rectangles 2½ inches long and five-eighths inch wide in each Petri-dish fungus culture. The seeds were then arranged on the glass just inside the edges of the remaining agar adjacent to the rectangular space, with one side of each seed in contact with the agar. The fungi were grown in all cases on corn-meal

[1] Received for publication Sept. 24, 1930; issued January, 1931.

[2] Reference is made by number (italic) to Literature Cited, p. 91.

agar. In each experiment one plate was inoculated with each fungus line. Throughout this paper a fungus culture arising from one isolation is called a line.

Seeds arranged along the perimeters of similar rectangles cut in sterile corn-meal agar served as controls. This sterile agar was part of the same batch of medium on which the inoculating fungi were grown. The uninoculated or control plates were made under the same conditions as the inoculated ones. They usually became overgrown with Mucor or Rhizopus because the seeds were not sterilized before inoculation, but this was not considered a disadvantage, because it made the moisture conditions in the uninoculated plates more nearly like those in the inoculated ones. The facts that the pine radicles in all uninoculated plates were entirely free from rot and that in spruce there was only an insignificant amount of radicle decay in one uninoculated plate suggest that this fungus was not much of a parasite under the conditions of these experiments. In each of the experiments numbered 100, 116, and 134, there were 25 uninoculated plates, and the probable error of the mean germination of these 25 has been calculated. The distribution of the figures from these uninoculated plates was approximately symmetrical. For each of the other experiments only five or six uninoculated plates were used.

Each plate in an experiment received the same quantity of seeds, which was determined by measure rather than by count. This, of course, introduced a certain small amount of error, probably partly compensated by the fact that very small seeds have a low germination percentage. In securing reliable results, the emphasis was placed on testing numerous lines of each important fungus and several replications for each line, rather than on extreme precision in the individual tests. The seed measure used for each plate in the experiments with *Pinus banksiana* Lamb. was large enough to hold on the average 100 seeds, in those with *P. resinosa* Ait. 75 seeds, and in those with *Picea engelmanni* Engelm. 200 seeds. After being seeded the plates were placed in a dark cupboard in the laboratory.

The experiments were continued until germination was practically complete, usually about 10 days after sprouting began and approximately 3 weeks after the seeds were sown on the plates. Results from plates that became dry before the ends of the experiments were discarded.

The method described exposed to infection not only the unruptured seeds but also radicles as they emerged from the seed coats. When the radicles were not attacked by the fungi, the seedlings developed fairly normal hypocotyls and cotyledons in the Petri dishes.

A second method (*26*) of inoculating radicles was to germinate the seeds on filter paper and to place the radicles of the seedlings in contact with rectangles of inoculated corn-meal agar in sterile Petri dishes. In this case, too, sterile agar was used for the controls. Ten seedlings served as a unit.

The principal fungi [3] tested by these methods were *Pythium ultimum*,[4] *Corticium vagum* B. and C. (*Rhizoctonia solani* Kühn), *Fusarium* spp., and *Botrytis* spp.

[3] In the experiments described in this paper the writer used Carl Hartley's cultures. The source and host of each of these cultures are given in an earlier paper (*29*, *Table I*). The writer wishes to thank Doctor Hartley for his kind suggestions, assistance, and criticisms regarding this investigation and this paper, especially the statistical portions.

[4] Formerly called *Pythium debaryanum* Hesse, but now *P. ultimum* seems the more probable name. However, no zoospores have been secured.

RESULTS OF THE PRESENT INVESTIGATION

The average number of seeds that germinated in units inoculated with the various fungi is shown in Table 1.

TABLE 1.—*Average number of seeds germinating in the various units*

[Fungi arranged in alphabetical order]

Fungi tested	Pinus banksiana				Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131
	Experiment No. 100	Experiment No. 110	Experiment No. 116	Experiment No. 120		
Botrytis spp. (small-sclerotium type)	67		60		50	
Botrytis cinerea	47		54	59	45	38
Cephalothecium roseum	36					
Corticium vagum (more virulent lines) [a]	46	52	56		50	16
Corticium vagum (less virulent lines) [a]	55	46	62	55	50	54
Fusarium spp. (exclusive of No. 906)	54	45	57	53	45	32
Fusarium acuminatum	59		54	56	42	
Fusarium culmorum group	56		57		38	
Fusarium discolor section	58		58	53	60	
Fusarium elegans group	54	54	57	51	46	
Fusarium moniliforme species and section	49	46	56	57	42	
Fusarium solani	74		61	51	44	32
Fusarium sporotrichioides	41	33	37		35	17
Fusarium vasinfectum	57		65			
Gibberella saubinetii	58	42	63	41	48	
Mucor racemosus			63	59		
Pestalozzia funerea (?)	43		53	50		
Phomopsis juniperovora	50		50		26	
Phycomycete (unidentified)	61					
Phytophthora sp.	48		60	36	45	
Phytophthora cactorum	61		44			
Pythiacystis citrophthora	42		56			
Pythium aphanidermatum	37		41		41	
Pythium artotrogus	58		61		43	
Pythium ultimum	46		50	48	45	39
Rhizoctonia potomacensis [b]	50					
Thielavia basicola	47		66			
Verticillium sp.			52			
Uninoculated	53	55	58	42	53	57

[a] Classified in accordance with apparent virulence to radicles.
[b] Probably a line of *Corticium vagum* according to H. A. Edson, who furnished it.

RADICLE DECAY

In Tables 2 to 8 are given the percentages of radicles of *Pinus banksiana*, *P. resinosa*, and *Picea engelmanni* that were decayed by each fungus line when tested by the first method described. Each percentage figure was weighted by the number of inoculated radicles, which in each plate equaled the number of seeds that germinated. In these tables the lines are arranged in decreasing order of apparent virulence, due consideration being given to a line and its reisolations. The average number of inoculated radicles in each experiment is given in Table 1 and the average radicle decay in each experiment in Table 8.

PYTHIUM ULTIMUM

In every experiment all the tested lines of *Pythium ultimum* were able to cause decay of young radicles. (Tables 2 and 8.) Most of them, including No. 767, which was only slightly virulent according to Hartley's (*11*) data, would probably be able to cause heavy germination loss in seed beds by killing young radicles soon after they emerge from the seed coats. There was no significant difference between the average radicle decay caused by lines isolated from coniferous hosts and by those isolated from nonconiferous hosts. (Table 8.) The

data were insufficient to prove whether there were real differences in host susceptibility, but there were some indications that the spruce radicles were more resistant than those of pine to certain lines.

The few experiments conducted by the second method failed to confirm the apparently greater resistance of spruce radicles to certain lines of *Pythium ultimum*. Although these results were based on inoculated units of only 10 seedlings, all the uninoculated radicles remained healthy, while the majority of the inoculated ones decayed. Two lines of *P. ultimum* killed 65 per cent of the 20 inoculated radicles of *Pinus banksiana;* six lines killed 97 per cent of the 60 of *P. resinosa*, and nine lines 89 per cent of the 110 of *Picea engelmanni*.

Other writers have found that Pythium can also cause germination loss to such unrelated hosts as sugar beets (*3, 25*), *Robinia pseudacacia* (*14*), and tobacco (*20*).

TABLE 2.—*Percentages of coniferous radicles decayed by various lines of Pythium ultimum*

Line No.	Pinus banksiana			Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131	Average
	Experiment No. 100	Experiment No. 116	Experiment No. 120			
810[a]	100	100	----	100	100	100
550	100	98	----	100	100	100
258	81	93	----	100	86	91
131[a]	----	95	100	100	94	97
408[a b]	100	92	----	----	----	96
338[a b]	----	100	96	100	12	85
295[a b]	100	100	----	89	20	81
767	100	100	----	90	27	83
296[a]	82	87	----	100	14	74
529[a]	58	35	----	36	----	42
743	2	23	----	----	93	40
Average uninoculated unit	0	0	0	0	.4	.1

[a] Lines isolated from nonconiferous hosts; no oospores have been observed for line 810.
[b] Reisolation of line No. 131.

OTHER PHYCOMYCETES

Two additional Phycomycetes (Tables 3 and 8), *Pythium aphanidermatum* Edson (Fitz)[5] and *Pythiacystis citrophthora* Sm. and Sm., showed as much apparent ability as *Pythium ultimum* to decay young radicles. However, despite the large amount of diagnostic work reported by Hartley (*11*), neither of them has been found occurring naturally in coniferous seed beds. *Phytophthora* sp. and *P. cactorum* (C. and L.) Sebrath appeared slightly parasitic to young radicles, whereas the other tested Phycomycetes gave no evidence of parasitism. According to Hartig (*8*), *P. fagi* kills seedlings of beech before they reach the surface of the soil.

[5] Called *Rheosporangium aphanidermatus* in a previous paper (*29, Table I*).

TABLE 3.—*Percentages of coniferous radicles decayed by various Phycomycetes*

Fungus	Line No.	Pinus banksiana			Pinus resinosa, experiment No. 134	Average
		Experiment No. 100	Experiment No. 116	Experiment No. 120		
Pythium aphanidermatum	[a] 351	100	97	----	100	99
	[a] [b] 430	51	100	----	----	77
Pythiacystis citrophthora	[a] 943	81	84	----	----	83
Phytophthora sp	[a] 843	0	58	----	----	32
	358	7	4	----	26	11
	372	0	----	8	4	4
Phytophthora cactorum	[a] 901	0	32	----	----	13
Pythium artotrogus	778	0	----	----	----	0
	821	0	0	----	0	0
	833	0	----	----	----	0
Unidentified Phycomycete	[a] 543	0	----	----	----	0
Mucor racemosus	[a] 927	----	0	0	----	0
Average uninoculated unit	----	0	0	0	0	0

[a] Lines isolated from nonconiferous hosts.
[b] Reisolation of line No. 351.
[c] Reisolation of line No. 372.

CORTICIUM VAGUM

Only the most virulent third (Tables 4 and 8) of the lines of *Corticium vagum* approximated the ability of *Pythium ultimum* to decay young radicles. Several of the *C. vagum* lines behaved as pure saprophytes, decaying none of the inoculated radicles. In many units sclerotia of *C. vagum* were produced on the exterior of the seeds. Even the more virulent *C. vagum* lines seemed less destructive than they did in Hartley's experiments (*11*). It is possible that the apparently greater importance of *C. vagum* in causing germination loss in nursery beds and in soil experiments is due to its ability to spread through soil more readily than *P. ultimum* does. A more important conclusion is that among the tested fungi these two are apparently the only important ones causing decay of radicles under natural conditions (*12*). In view of the fact that some lines of *C. vagum* are known to cause disease only at low temperatures, it is possible that experiments conducted at temperatures lower than the ordinary laboratory ones might result in the production of more radicle decay by this fungus. Balls (*1*) found that the "sore-shin" fungus vigorously attacked germinating cottonseeds at 20° C., but that it caused only slight superficial lesions at 33° and no injury at 37°.

On the whole, *Corticium vagum* decayed fewer radicles by the second than by the first method of inoculation. Six of the more virulent lines killed 73 per cent of the 60 inoculated radicles of *Pinus resinosa*, and seven killed 36 per cent of the 70 inoculated radicles of *Picea engelmanni*. Three and five of the less virulent lines killed none of the inoculated radicles of *Pinus banksiana* and *P. resinosa*, respectively, while nine of them killed 17 per cent of the 110 inoculated radicles of *Picea engelmanni*.

Rhizoctonia seems also to vary in its virulence to other hosts, as it is known to cause germination loss in tobacco seed beds (*20*) and to kill germinating seeds of cotton (*7*), but it did not prevent bean seeds from germinating (*13*).

TABLE 4.—*Percentages of coniferous radicles decayed by various lines of Corticium vagum*

Line No.	Pinus banksiana				Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131	Average
	Experiment No. 100	Experiment No. 110	Experiment No. 116	Experiment No. 120			
More virulent lines:							
183 [a]	100		98		80		94
187 [a]	69		68		100		79
213 [a]	99		65		100	25	83
331 [a b]	100	76	13		100		74
340 [a b]	82	45	75		92		71
332 [c]	100		91		100		98
333 [c]	52		95		96		85
329 [c]		54	94		100		75
147 [d]	41	8	40				33
343 [c d]			2				2
746	100		82				88
747 [d e]	92		29		7		37
Less virulent lines:							
240			50		13		35
205	0		66		0		24
50			23	14			19
341 [a f]	32	26	78		20	4	32
233 [a f]	8	10	50		5	30	23
230 [a]		2	1		2		2
330 [a f]	0		2		0	0	1
761	11	0		16	19		11
724	0		2		32		10
380	10		1				5
361	8		1		4		4
362	2			0			1
723				0			0
721			0				0
552			0				0
365	0		0				0
363	0	0	0				0
189 [a]	0		0				0
186 [a]	0		0				0
Average uninoculated unit	0	0	0	0	0	.4	.1

[a] Lines isolated from nonconiferous hosts.
[b] Reisolation of line No. 213.
[c] Reisolation of line No. 147.
[d] Included because of relation to other lines in the more virulent group.
[e] Duplicate of line No. 746.
[f] Reisolation or duplicate of line No. 230.

FUSARIUM SPP.

Fusarium sporotrichioides, which has never yet been isolated from damped-off coniferous seedlings (*11*), was the only species of Fusarium (Tables 5 and 8) that approached *Pythium ultimum* in ability to decay radicles. One other Fusarium culture, which was omitted from the table because it was later found to contain two species (*F. moniliforme* and *F. discolor* [6]), also showed decided ability to attack radicles.

Some lines of the *moniliforme* group of Fusarium also gave some evidence of ability to decay radicles. Many of these lines were 7 years old when they were used in these experiments and had lost their pigment-forming capacity. This fungus is also known to cause root rot of corn (*2, 34*) and under certain conditions to inhibit germination (*22*.) *Fusarium discolor sulphureum* and *F. arthrosporioides* also showed consistent ability to attack radicles.

The other Fusarium species, even those that were fairly parasitic to older seedlings (*29*), either showed little ability to attack radicles or behaved as pure saprophytes. *Fusarium acuminatum*, *F. ventricosum*, *F. orthoceras*?, *F. avenaceum*, *F. hyperoxysporum*, *F. coeruleum*,

[6] Determined by Helen Johann.

F. oxysporum, *F. radicicola*, 3 lines of the *moniliforme* section, 3 lines of the *discolor* section, 1 line of *Gibberella saubinetii*, 5 lines of the *F. elegans* group, 2 lines of the *F. culmorum* group, 2 lines of the *F. solani* group, and 2 unidentified species of Fusarium showed consistent lack of ability to attack radicles. The remaining lines were sometimes parasitic and at other times nonparasitic to young radicles. These results confirm the earlier conclusions based on soil inoculation tests with *F. moniliforme* and *F. ventricosum* (*12*).

TABLE 5.—*Percentages of coniferous radicles decayed by various lines of Fusarium, Gibberella, and Nectria*

Fungus	Line No.	Pinus banksiana				Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131	Average
		Experiment No. 100	Experiment No. 110	Experiment No. 116	Experiment No. 120			
Fusarium sporotrichioides	* 906	100	54	84		86	100	84
Fusarium discolor sulphureum	* 936	19		16				17
Fusarium moniliforme species and section.	* 948	59		4		86		30
	247	53		0				28
	266	6			8	17		10
	* 949	0		0		38		10
	* 308	0		2		4		2
	* 910	7		0		0		2
	273	0	2	2				1
	* 947	0		0		4		1
	239			3	0	0		1
	249	0	5		0	0		1
	251			0	0	0		0
	260	0		0		0		0
	265	0		0		0		0
Fusarium arthrosporioides	* 908	2		3		12		4
Fusarium trichothecioides	* 941	0		0		12		4
Fusarium martiella section	268	0	0	4		8		3
Fusarium between discolor and roseum groups	* 946	0		4		10		3
Gibberella saubinetii	* 916		0	3	0	9		3
	* 933	0		0		0		0
	* 915	9		0		6		5
	779	0	4	5		0		2
	* 913				3	0		2
Fusarium elegans group	370	0		0				0
	736	0		0		0		0
	* 904	0		0		0		0
	* 909			0	0			0
	* 912	0			0			0
Fusarium culmorum group	* 917	0		9		0		3
	* 929	0		0				0
	* 557	0		0				0
Fusarium solani	* 940			0	0	9		3
	* 905			0	0		0	0
	262	0		0				0
Fusarium vasinfectum	* 928	0		5				3
	* 942	0		0				0
Fusarium martii	* 914	4	0	0				1
Fusarium eumartii	* 937	0		1		0		1
Nectria ipomoeae	* 931	2		0				1
Fusarium acuminatum	283	0		0		0		0
	508	0			0	0		0
	* 932	0		0				0
Fusarium discolor section	367			0	0			0
	* 518	0		0		0		0
	935	0		0		0		0
Fusarium ventricosum	259	0		0				0
Fusarium orthoceras (?)	357			0	0			0
Unidentified Fusarium	416	0	0	0				0
Unidentified Fusarium	446	0				0		0
Fusarium avenaceum	* 911	0		0				0
Fusarium hyperoxysporum	* 930	0		0		0		0
Fusarium coeruleum	* 934	0		0		0		0
Fusarium oxysporum	* 938	0	0	0				0
Fusarium radicicola	* 939			0	0			0
Average uninoculated unit		0	0	0	0	0	.4	.1

* Lines isolated from nonconiferous hosts.

On the whole, the Fusarium lines seemed to cause more decay of radicles of *Pinus resinosa* than of those of *P. banksiana*, but further experiments are necessary to determine whether this difference is constant. A species of Fusarium has been reported as causing a blight of maple (*6*), and others have been listed from such forest-tree seeds as hickory, horsechestnut, and walnut (*4*).

The results of the inoculation of coniferous radicles with *Fusarium* spp. by the second method confirmed the ability of *F. sporotrichioides* and *F. arthrosporioides* to decay radicles but gave no evidence of such ability for any of the other lines. *F. sporotrichioides* caused 70 per cent of the radicles of *Pinus banksiana* and 50 per cent of those of *Picea engelmanni* to decay, and *F. arthrosporioides* decayed 70 per cent of those of the pine but none of those of the spruce. One line each of the *F. elegans* group and of *Gibberella saubinetii* and two lines of the *F. moniliforme* group decayed no radicles of *Pinus banksiana*. Three lines of the *F. moniliforme* group and one of *G. saubinetii* decayed no radicles of *P. resinosa*. Five lines of the *F. moniliforme* group, two of *G. saubinetii*, two of the *F. elegans* group, one each of the *F. discolor* group, *F. martii*, *F. hyperoxysporum*, and one line of a taxonomic position between the *F. discolor* and *F. roseum* groups were harmless to the radicles of *Picea engelmanni*.

Botrytis Spp.

The small-sclerotium type of Botrytis (Tables 6 and 8) gave no evidence of ability to decay radicles. The large-sclerotium lines, commonly called *Botrytis cinerea*, were more or less parasitic to young radicles, but appeared far less parasitic to these than to stems (*29*). In the one experiment in which *Pinus banksiana* radicles were inoculated with line No. 944 by the second method, 30 per cent of the radicles were decayed. *B. cinerea* has been reported as attacking forest-tree seeds such as chestnut (*21*) and horsechestnut (*24*). It also causes a disease of pistillate catkins of birch (*23*).

Miscellaneous Imperfect Fungi

Every tested line of *Phomopsis juniperovora* (Tables 7 and 8) caused some radicle decay in every experiment in which it was tested. None of the other tested imperfect fungi showed any evidence of causing radicle decay.

Table 6.—*Percentages of coniferous radicles decayed by Botrytis spp.*

Fungus	Line No.	Pinus banksiana			Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131	Average
		Experiment No. 100	Experiment No. 116	Experiment No. 120			
Botrytis cinerea	[a] 944	22	32	.....	33	0	22
	[a] 920	10	11	0	0	.....	6
	[a] 924	10	0	.....	0	.....	4
	470	4	0	.....	2	.....	2
Botrytis spp. (small-sclerotium type)	[a] 921	.....	0	0	4	.....	1
	[a] 922	0	0	.....	0	.....	0
	[a] 923	.....	0	.....	0	.....	0
Average uninoculated unit	.....	0	0	0	0	.4	.1

[a] Lines isolated from nonconiferous hosts.

TABLE 7.—*Percentages of coniferous radicles decayed by miscellaneous imperfect fungi*

Fungus	Line No.	Pinus banksiana			Pinus resinosa, experiment No. 134	Average
		Experiment No. 100	Experiment No. 116	Experiment No. 120		
Phomopsis juniperovora	860	38	42		18	35
	864	14	14		81	23
	865	15	12		20	15
	867	11	8			9
Cephalothecium roseum	[a] 288	0				0
Verticillium sp.	[a] 399		0			0
Thielavia basicola	[a] 428	0	0			0
Pestalozzia funerea (?)	968	0	0	0		0
Rhizoctonia potomacensis	[a] 881	0				0
Average uninoculated unit		0	0	0	0	0

[a] Lines isolated from nonconiferous hosts.

COMPARISON OF DECAY CAUSED BY THE DIFFERENT FUNGI

A comparison of the average percentages of coniferous radicle decay caused by the different fungi is shown in Table 8.

TABLE 8.—*Average percentages of coniferous radicle decay caused by the different fungi*

[Fungi listed in alphabetical order. The results for species of which only one line was tested are given in Tables 3, 5, and 7]

Fungi tested	Pinus banksiana				Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131	Average
	Experiment No. 100	Experiment No. 110	Experiment No. 116	Experiment No. 120			
Botrytis spp. (small-sclerotium type)	0		0		0		0
Botrytis cinerea	11		7	0	8	0	7
Corticium vagum (more virulent lines):							
All lines	85	50	63		83	25	71
Lines from coniferous hosts	78	34	60		68		64
Lines from nonconiferous hosts	92	60	66		95	25	79
Line No. 213 and its reisolations	95	60	53		97	25	75
Line No. 147 and its reisolations	68	34	64		98		65
Line No. 746 and its duplicate	96		54		7		55
Corticium vagum (less virulent lines):							
All lines	5	7	16	7	10	14	11
Lines from coniferous hosts	4	0	14	7	13		9
Lines from nonconiferous hosts	7	13	20		7	14	13
Line No. 230 and its reisolations	12	13	29		7	14	16
Fusarium spp. (exclusive of No. 906)	2	1	1	1	6	0	2
Fusarium acuminatum	0		0	0	0		0
Fusarium culmorum group	0		2		0		1
Fusarium discolor section	0		0	0	0		0
Fusarium elegans group	1	4	1	2	1		1
Fusarium moniliforme species and section	8	3	1	2	1		6
Fusarium solani	0		0	0	9	0	1
Fusarium vasinfectum	0		2				1
Gibberella saubinetii	0	0	2	0	4		2
Phomopsis juniperovora	19		18		32		21
Phytophthora spp.	3		32	8	15		15
Pythium aphanidermatum	74		99		100		90
Pythium artotrogus	0		0		0		0
Pythium ultimum:							
All lines	83		84	98	89	67	82
Lines from coniferous hosts	74		79		97	76	81
Lines from nonconiferous hosts	90		87	98	85	59	83
Line No. 131 and its reisolations	100		97	98	96	54	89
Uninoculated	0	0	0	0	0	.4	.1

SPROUTING DECREASE

The decay of unruptured seeds is, of course, more difficult to determine quantitatively than is the decay of radicles. The only way that it seemed practicable to estimate the amount of seed decay in these experiments was to compare the number of sprouted seeds, indicated by rupturing visible with a hand lens, in each inoculated unit with that in the average uninoculated unit in the same experiment. (Tables 9 to 16.) The normal variability in the germination of coniferous seeds served to introduce a serious source of error in this method of determination. Fortunately, the distributions in the 75 uninoculated units of the three large experiments were practically symmetrical.

In examining the significance of the average differences between inoculated and uninoculated units (next to the last columns in Tables 9 to 15), the procedure used was to determine the probability of an accidental difference in the direction and of the magnitude observed. This was done by the method outlined by Fisher (*5, p. 106*), halving the probability values given at the top of his table of t[7] (*5, p. 137*) in accordance with his instructions (*5, p. 107*). The complement of this halved value was taken as the probability that the observed difference was due to the inoculation. When the difference was an apparent increase in the percentage sprouting, the probability obtained as above was the probability that the inoculation resulted in increased sprouting percentage, and its complement was taken as the probability that the fungus actually decreased germination. In cases of apparent decreases, therefore, the probability that inoculation resulted in decrease (last columns of Tables 9 to 15) is the complement of the probability of accidental occurrence of so large a decrease; for apparent increases it is identical with the probability of accidental occurrence of such an increase.

It seems at first thought impossible to speak of probability of decrease in cases in which there was an apparent increase. As a matter of fact, there is no inconsistency; while the inoculated units in such cases gave higher sprouting percentages than the uninoculated, there is still a chance that the units chosen in advance for inoculation happened to be especially good ones and would have shown a still higher sprouting percentage if they had not been inoculated. Because the available data indicate an increase, there is less than an even chance in such cases that the inoculation caused a decrease, and the probability of decrease is less than 0.5.

The mathematical interpretation can go only far enough to express the probability that the inoculated units under consideration belonged

[7] Fisher's notation is different from that employed in most American literature, particularly in that he uses n' in place of n, and n in place of $(n-1)$. The term "t" used by Fisher is the ratio of the mean of a sample to the standard error computed from the sample. His table of t gives the probability of the mean of a sample departing so far from zero in either the positive or negative direction when the true mean of the population sampled is zero. To use a more common notation, when M is the mean, Σd^2 the sum of the squared deviations, and n the number of items, $t=\frac{M}{\sqrt{\Sigma d^2/n\ (n-1)}}$. For examining the significance of the difference between means of two samples, t is the ratio of the difference to its standard error, obtained as follows:

$$t=\frac{M_1-M_2}{\sqrt{\left(\frac{1}{n_1}+\frac{1}{n_2}\right)\frac{\Sigma d^2_1+\Sigma d^2_2}{n_1+n_2-2}}}$$

For a mean based on 10 items, Fisher's table of t is entered on line 9; for a difference between a mean based on 10 items and a mean based on 8 items, the proper line in the table of t is $(10-1)+(8-1)$ or the sixteenth line.

to a population with a lower mean than the uninoculated units. The most logical assumption appears to be that any decreases due to the inoculation were the result of the invasion and killing of the seeds by the fungus added. It is possible, however, that the effect of the inoculation was due to toxins produced by the fungus, to exhaustion of the oxygen in the Petri dish, or to some other indirect influence. The probability computations give no information as to why the inoculated units differed from those uninoculated.

In Tables 9 to 14, inclusive, the lines are arranged in decreasing order of apparent virulence to radicles. The average increase or decrease in sprouting percentage is based on germination in the average uninoculated unit. (Table 1.) The absolute difference between the amount of sprouting in the mean uninoculated unit and that in each individual inoculated unit was calculated; then the percentage sprouting decrease was calculated from this difference and the germination in the mean inoculated unit. The results for the average inoculated units are given in Table 15.

Pythium ultimum

The absolute differences between the germination in the mean uninoculated unit and that in the mean unit inoculated with *Pythium ultimum* lines were not great for each experiment, but they were consistent enough to establish the fact that this fungus can kill some seeds which, so far as could be determined externally, had not started to germinate. For the experiments for which probable errors could be calculated, the weighted average difference between the sprouting of the average uninoculated unit and that of the average inoculated unit was 9.13 ± 1.05.

There was no significant difference between the average amount of reduction in sprouting caused by *Pythium ultimum* lines isolated from coniferous hosts and those from nonconiferous hosts.

Further and more extensive experiments would be necessary to establish definitely the relative virulence of the different lines. As might be expected, the addition of a parasite, an important variable factor, resulted in increasing variability as well as in decreasing sprouting. The coefficients of variability for the number of seeds germinating in the *Pythium ultimum* units were 18.7 ± 3.1 per cent in experiment No. 100, 11.8 ± 1.7 per cent in experiment No. 116, and 21.4 ± 3.6 per cent in experiment No. 134, as compared with 9.6 ± 0.92 per cent, 11.7 ± 1.12 per cent, and 10.3 ± 0.98 per cent for the uninoculated units in the respective experiments. For this reason greater differences in results are required to establish definitely the significance of the differences between the units inoculated with different fungi than between inoculated and uninoculated units.

It is significant that all the average percentage figures in next to the last column of Table 9 are negative and that the probabilities of sproutings being less in inoculated than in uninoculated units are more than 0.80 for all lines except No. 131. The average percentage apparent sprouting decrease for all lines was 17, and for these same lines for all experiments except No. 120, in which there was an unexplained lowering of germination of uninoculated units, the probabilities of sproutings being less in inoculated than in uninoculated units are more than 0.98. (Table 15.)

TABLE 9.—*Comparison between the sprouting of coniferous seeds in each unit inoculated with Pythium ultimum and that in the average uninoculated unit in the same experiment*

Line No.	Apparent increase (+) or decrease (−) in sprouting percentage						Probability that sprouting will be less in inoculated than in uninoculated units
	Pinus banksiana			Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131	Average[a]	
	Experiment No. 100	Experiment No. 116	Experiment No. 120				
810[b]	−21	−11		+14	−16	−9	0.84
550	−14	−36		−14	−30	−24	.99
258	−12	−24		−.6	−51	−22	.94
131[b]		+2	−0.9	−29	+7	−5	.71
408[b c]	−4	−13				−9	.85
338[b c]		−13	+25	−34	−54	−19	.83
295[b c]	+16	−18		−31	−32	−16	.88
767	+3	+0.7		−10	−28	−9	.85
296[b]	−40	−8		−44	−51	−36	.98
529[b]	−29	−22		−.6		−14	.88
743	−23	−18			−25	−22	.99+

[a] It would probably be preferable to use the geometric means, which would result in greater average decreases; but except in cases of extreme variability, as with line 258, the difference would be slight.
[b] Lines isolated from nonconiferous hosts.
[c] Reisolation of line No. 131.

OTHER PHYCOMYCETES

Pythium aphanidermatum and *Pythiacystis citrophthora* caused apparent sprouting decrease in every test with them (Tables 10 and 15), whereas the other Phycomycetes either caused no apparent sprouting decrease or caused it only part of the time.

TABLE 10.—*Comparison between the sprouting of coniferous seeds in each unit inoculated with a miscellaneous Phycomycete and that in the average uninoculated unit in the same experiment*

Fungus	Line No.	Apparent increase (+) or decrease (−) in sprouting percentage					Probability that sprouting will be less in inoculated than in uninoculated units
		Pinus banksiana			Pinus resinosa, experiment No. 134	Average	
		Experiment No. 100	Experiment No. 116	Experiment No. 120			
Pythium aphanidermatum	[a] 351	−34	−34		−23	−30	0.99
	[b] 430	−27	−24			−26	.98
Pythiacystis citrophthora	[a] 943	−21	−3			−12	.80
Phytophthora sp	[c] 843	−4	+8			+2	.39
	358	+5	−1		−21	−6	.74
	372	−32		−15	−12	−20	.96
Phytophthora cactorum	[a] 901	+14	−24			−5	.59
Pythium artotrogus	778	+7				+7	
	821	+1	+6		−19	−4	.68
	833	+20				+20	
Unidentified Phycomycete	[a] 543	+14				+14	
Mucorracemosus	[a] 927		+9	+39		+24	.18

[a] Lines isolated from nonconiferous hosts.
[b] Reisolation of line No. 351.
[c] Reisolation of line No. 372.

Corticium vagum

Approximately 83 per cent of the *Corticium vagum* lines classified as more virulent to radicles (Table 11) caused some apparent average sprouting decrease, but only a little more than one-third of those for which probabilities could be computed had probabilities of more than 0.80 of the sproutings being less in inoculated than in uninoculated units. The average apparent decrease in sprouting percentage was 9 (Table 15), but the probabilities of sproutings being less in inoculated than in uninoculated units varied greatly for the different experiments. For the experiments for which probable errors could be calculated, the weighted average difference between the sprouting in the average uninoculated unit and that of the average inoculated unit was 5.6 ± 0.87.

Approximately one-third of the lines of *Corticium vagum* classified as less virulent to radicles (Table 11) caused an apparent average decrease in sprouting, but the variability was so high that only one of these lines had a probability of more than 0.80 of sproutings being less in inoculated than in uninoculated units.

Table 11.—*Comparison between the sprouting of coniferous seeds in each unit inoculated with Corticium vagum and that in the average uninoculated unit in the same experiment*

Line No.	Apparent increase (+) or decrease (−) in sprouting percentage							Probability that sprouting will be less in inoculated than in uninoculated units
	Pinus banksiana				Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131	Average	
	Experiment No. 100	Experiment No. 110	Experiment No. 116	Experiment No. 120				
More virulent lines:								
183 [a]	−23		+6		−27		−15	0.86
187 [a]	−32		−18		−17		−22	.98
213 [a]	+28		−.1		−10	−72	−14	.73
331 [a b]	+11	+8	−17		−14		−3	.65
340 [a b]	−19	+19	−.1		−10		−3	.63
332 [c]	+11		−20		+20		+4	.39
333 [c]	−38		−6		−6		−17	.87
329 [c]		−16	−13		(?)		−15	.97
147	−8	−34	+20				−7	.65
343 [c]			−18				−18	
746	−36		+14				−11	.64
747 [c d]	−25		+30		+7		+4	.41
Less virulent lines:								
240			+8		−16		−4	.61
205	+28		+13		−16		+8	.30
50			−11	+20			+5	.40
341 [a e]	−19	−16	−13		+3	−11	−11	.98
233 [a e]	−.6	−28	+4		−21	+18	−6	.74
230 [a]		−23	+27		+3		+2	.45
330 [a e]	+11		+6		+3	−21	−.3	.52
761	−17	−10		+34	−19		−3	.59
724	+5		+8		−6		+2	.34
380	+14		+22				+18	.07
361	+16		+22		+7		+15	.04
362	−21			+32			+6	.43
723				+32			+32	
721			+4				+4	
552			+20				+20	
365	+11		+2				+7	.18
363	+18	−.9	+.7				+6	.21
189 [a]	−21		+4				−9	.70
186 [a]	+20		+.7				+10	.24

[a] Lines isolated from nonconiferous hosts.
[b] Reisolation of line No. 213.
[c] Reisolation of line No. 147.
[d] Duplicate of line No. 746.
[e] Reisolation or duplicate of line No. 230.

FUSARIUM SPP.

In general, the Fusarium lines caused little apparent sprouting decrease of *Pinus banksiana*. (Table 12.) In some of the units of *P. resinosa* there was decided evidence of sprouting decrease. *Fusarium sporotrichioides* gave decided evidence of ability to cause sprouting decrease in every experiment, fully equaling that of the strongly parasitic *Pythium ultimum* lines. Irrespective of the magnitude of the sprouting decreases, the mere fact that the units inoculated with it always sprouted less than the uninoculated units is evidence that the fungus caused the decrease. A mixed culture of *F. moniliforme* and *F. discolor* also showed decided evidence of ability to decrease sprouting. More than half of the lines belonging to the *moniliforme* section of Fusarium have probabilities of more than 0.80 of sproutings being less in the inoculated than in the uninoculated units. The probabilities vary for different experiments. (Table 15.) Eleven other Fusarium lines belonging to different species have probabilities of more than 0.80 of sproutings being less than in the inoculated units.

TABLE 12.—*Comparison between the sprouting of coniferous seeds in each unit inoculated with Fusarium, Gibberella, and Nectria and that in the average uninoculated unit in the same experiment*

Fungus	Line No.	Apparent increase (+) or decrease (−) in sprouting percentage							Probability that sprouting will be less in the inoculated than in uninoculated units
		Pinus banksiana				Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131	Average	
		Experiment No. 100	Experiment No. 110	Experiment No. 116	Experiment No. 120				
Fusarium sporotrichioides	* 906	−23	−40	−36	.....	−34	−70	−41	0.99+
Fusarium discolor sulphureum	* 936	−2	.....	−11	.....	.....	.....	−7	.82
	* 948	−17	.....	−6	.....	−23	.....	−15	.95
	247	+11	.....	−11	.....	.....	.....	0	.....
	266	−8	.....	.....	+46	−14	.....	+8	.36
	* 949	+5	.....	−13	.....	−27	.....	−12	.84
	* 308	−17	.....	+.7	.....	−14	.....	−10	.89
Fusarium moniliforme species and section	* 910	−16	.....	−5	.....	−16	.....	−12	.96
	273	−6	−3	+8	.....	.....	.....	−.3	.53
	* 947	−27	.....	−6	.....	−8	.....	−14	.92
	239	.....	.....	+11	+25	−27	.....	+3	.43
	249	−4	−28	.....	+23	−48	.....	−14	.79
	251	.....	.....	+13	+44	−6	.....	+17	.18
	260	−6	.....	+2	.....	−46	.....	−17	.82
	265	−2	.....	−18	.....	−10	.....	−10	.92
Fusarium arthrosporioides	* 908	−4	.....	+2	.....	−53	.....	−18	.80
Fusarium trichothecioides	* 941	−27	.....	−3	.....	−21	.....	−17	.93
Fusarium martiella section	268	−2	−32	−11	.....	−.6	.....	−11	.89
Fusarium between discolor and roseum groups	* 946	+1	.....	−6	.....	−42	.....	−16	.82
Gibberella saubinetii	* 916	.....	−23	+.7	−3	−17	.....	−11	.93
	* 933	+9	.....	+18	.....	−2	.....	+8	.15
	* 915	−19	.....	−13	.....	−10	.....	−14	.98
	779	−17	−1	−1	.....	−38	.....	−14	.87
	* 913	.....	.....	.....	+18	−14	.....	+2	.46
Fusarium elegans group	370	+11	.....	−5	.....	.....	.....	+3	.39
	736	+22	.....	+8	.....	−.6	.....	+10	.14
	* 904	+9	.....	−6	.....	−6	.....	−2	.57
	* 909	.....	.....	+8	+27	.....	.....	+18	.15
	* 912	−.6	.....	.....	+13	.....	.....	+4	.27
Fusarium culmorum	* 917	−.6	.....	−18	.....	−29	.....	−16	.91
	* 929	+13	.....	−11	.....	.....	.....	+1	.48
	557	+5	.....	+15	.....	.....	.....	+10	.15
Fusarium solani	* 940	.....	.....	−3	+.14	−17	.....	−7	.68
	* 905	.....	.....	+8	+37	.....	−44	+.3	.50
	202	+39	.....	+11	.....	.....	.....	+25	.17
Fusarium vasinfectum	* 928	+5	.....	+8	.....	.....	.....	+7	.08
	* 942	+7	.....	+18	.....	.....	.....	+13	.13

* Lines isolated from nonconiferous hosts.

TABLE 12.—*Comparison between the sprouting of coniferous seeds in each unit inoculated with Fusarium, Gibberella, and Nectria and that in the average uninoculated unit in the same experiment*—Continued

Fungus	Line No.	Apparent increase (+) or decrease (−) in sprouting percentage							Probability that sprouting will be less in the inoculated than in uninoculated units
		Pinus banksiana				Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131	Average	
		Experiment No. 100	Experiment No. 110	Experiment No. 116	Experiment No. 120				
Fusarium martii	[a] 914	+7	−3	+0.7				+2	0.28
Fusarium eumartii	[a] 937	−6		+16		+3		+4	.30
Nectria ipomoeae	[a] 931	−4		+6				+1	.43
Fusarium acuminatum	283	+11		−13		−16		−6	.72
	508	+7			+32	−29		+3	.44
	[a] 932	+16		−1				+8	.26
Fusarium discolor section	367			+2	+25			+14	.22
	[a] 518	+11		−18		+3		−1	.54
	[a] 935	+5		+18		+22		+15	.05
Fusarium ventricosum	299	+18		−22				−2	.54
Fusarium orthoceras (?)	357			−5	+46			+21	.29
Unidentified Fusarium	416	−.6	−17	−3				−7	.85
Unidentified Fusarium	446	+22				−10		+6	.39
Fusarium avenaceum	[a] 911	+9		+.7				+5	.22
Fusarium hyperoxysporum	[a] 930	−.6		+6		+7		+4	.12
Fusarium coeruleum	[a] 934	+22		+4		+24		+17	.06
Fusarium oxysporum	[a] 938	+36	−28	+13				+7	.37
Fusarium radicicola	[a] 939			−15	+13			−1	.53
Average inoculated unit exclusive of line No. 906		+3	−17	−1	+25	−15	−44	−1	

[a] Lines isolated from nonconiferous hosts.

BOTRYTIS SPP.

The small-sclerotium type of Botrytis and three lines of *Botrytis cinerea* caused no apparent average decrease in sprouting percentage. (Table 13.) The other two lines of *B. cinerea* not only caused a large apparent average decrease in sprouting percentage but also had high probabilities of sproutings being less in inoculated than in uninoculated units. In three experiments (Table 15) there were high probabilities of sproutings being less in the inoculated units.

TABLE 13.—*Comparison between the sprouting of coniferous seeds in each unit inoculated with Botrytis spp. and that in the average uninoculated unit in the same experiment*

Fungus	Line No.	Apparent increase (+) or decrease (−) in sprouting percentage						Probability that sprouting will be less in inoculated than in uninoculated units
		Pinus banksiana			Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131	Average	
		Experiment No. 100	Experiment No. 116	Experiment No. 120				
Botrytis cinerea	[a] 944	−17	−34		−16	−33	−25	0.99
	[a] 920	−44	−1	+4	−72		−28	.89
	[a] 924	+11	−8		+7		+3	.33
	470	−.6	−5		+7		+.4	.46
Botrytis spp. (small-sclerotium form)	[a] 921		+13	+75	−6		+27	.20
	[a] 922	+26	+9		−25		+3	.43
	[a] 923		−1		+11		+5	.28

[a] Lines isolated from nonconiferous hosts.

Miscellaneous Imperfect Fungi

Phomopsis juniperovora caused considerable apparent sprouting decrease and had high probabilities of sprouting's being less in the inoculated than in the uninoculated units. (Tables 14 and 15.) *Cephalothecium roseum*, *Verticillium* sp., *Pestalozzia funerea* (?), and *Rhizoctonia potomacensis* all caused some apparent sprouting decrease.

Table 14.—*Comparison between the sprouting of coniferous seeds in each unit inoculated with miscellaneous imperfect fungi and that in the average uninoculated unit in the same experiment*

Fungus	Line No.	Apparent increase (+) or decrease (−) in sprouting percentage					Probability that sprouting will be less in inoculated than in uninoculated units
		Pinus banksiana			Pinus resinosa, experiment No. 134	Average	
		Experiment No. 100	Experiment No. 116	Experiment No. 120			
Phomopsis juniperovora	850	−12	−17		−47	−25	0.93
	864	−17	−11		−70	−33	.89
	865	+3	−15		−34	−15	.85
	867	+1	−8			−4	.74
Cephalothecium roseum	[a] 288	−32				−32	
Verticillium sp.	[a] 399		−10			−10	
Thielavia basicola	[a] 428	−12	+15			+2	.45
Pestalozzia funerea (?)	968	−19	−8	+18		−3	.59
Rhizoctonia potomacensis	[a] 881	−6				−6	

[a] Lines isolated from nonconiferous hosts.

Comparison of Sprouting Decreases Caused by the Different Fungi

A comparison of the sprouting decreases caused by the different fungi is shown in Table 15.

TABLE 15.—*Comparison between the sprouting of coniferous seeds in the average inoculated unit and that in the average uninoculated unit*

[Fungi listed in alphabetical order. The results for species of which only one line was tested are given in Tables 10, 12, and 14]

Fungi tested	Apparent increase (+) or decrease (−) in sprouting percentage							Probability that sprouting will be less in inoculated than in uninoculated units					
	Pinus banksiana				Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131	Average	Pinus banksiana				Pinus resinosa, experiment No. 134	Picea engelmanni, experiment No. 131
	Experiment No. 100	Experiment No. 110	Experiment No. 116	Experiment No. 120				Experiment No. 100	Experiment No. 110	Experiment No. 116	Experiment No. 120		
Botrytis spp. (small-sclerotium type)	+26		+4		−7		+4			0.29		0.62	
Botrytis cinerea	−13		−7	+40	−16	−33	−7	0.82		.80	0.23	.83	
Corticium vagum (lines more virulent to radicles):													
All lines	−13	−6	−2		−7	−72	−9	.95	0.68	.66		.89	
Lines from coniferous hosts	−19	−25	+1		+7		−7	.95	.89	.45		.23	
Lines from nonconiferous hosts	−7	+14	−6		−16	−72	−11	.71	.12	.86		.99+	
Line 213 and its reisolations	+7	+14	−6		−11	−72	−7	.33	.12	.80		.99	
Line 147 and its reisolations	−12	−25	−7		+7		−9	.76	.89	.81		.34	
Line 746 and its duplicate	−31		+22		+7		−2	.95		.11			
Corticium vagum (lines less virulent to radicles):													
All lines	+3	−16	+7	+30	−7	−5	+2	.27	.99	.01	0	.95	0.65
Lines from coniferous hosts	+7	−6	+9	+30	−10		+7	.14	.80	.01	0	.95	
Lines from nonconiferous hosts	−2	−22	+5		−5	−5	−4	.53	.99	.19		.68	.65
Line 230 and its reisolations	−3	−22	+6		−3	−5	−5	.62	.99	.26		.68	.65
Fusarium acuminatum	+11		−7	+32	−23		+1	.03		.78		.91	
Fusarium culmorum group	+6		−5		−29		−4	.14		.67			
Fusarium discolor section	+8		+1	+25	+13		+9	.11		.48	.20		
Fusarium elegans group	+1	−1	−2	+19	−14		+1	.45		.71	.02	.90	
Fusarium moniliforme species and section	−8	−16	−2	+35	−22		−6	.99	.79	.74	.01−	.99+	
Fusarium solani	+39		+5	+19	−17	−44	+4			.18	.25		
Fusarium vasinfectum	+6		+13				+10	.05		.12			
Gibberella saubinetii	+9	−23	+9	−3	−10		−3			.24		.80	
Phomopsis juniperovora	−6		−13		−50		−21	.85		.99+		.98	
Phytophthora sp	−10		+4	−15	−17		−9	.77		.27		.91	
Pythium aphanidermatum	−31		−29		−23		−28	.96		.95			
Pythium artotrogus	+9		+6		−19		+3	.13					
Pythium ultimum:													
All lines	−14		−14	+12	−17	−31	−17	.98		.99+	.26	.98	.99+
Lines from coniferous hosts	−12		−19		−8	−33	−19	.95		.96		.91	.99
Lines from nonconiferous hosts	−16		−10	+12	−21	−29	−16	.91		.99+	.26	.97	.97
Line 131 and its reisolation	+6		−11	+12	−31	−26	−13	.33		.96	.26	.99+	.86

RELATION BETWEEN RADICLE DECAY AND SPROUTING DECREASE

Table 16 summarizes the relationship between radicle decay and sprouting decrease. With the exception of the less virulent lines of *Corticium vagum*, in more than 70 per cent of the units radicle decay was accompanied by apparent sprouting decrease.

However, for both *Pythium ultimum* and *Corticium vagum* the lines that caused the most apparent decrease in sprouting were not always the ones that decayed the most radicles. *Pythium aphanidermatum*, *Pythiacystis citrophthora*, and *Fusarium sporotrichioides* caused apparent sprouting decrease and radicle decay in every test with them.

Botrytis cinerea, line No. 944, which seemed most virulent to young radicles, caused some apparent sprouting decrease in each of the four experiments in which it was tested. Line No. 920, which was the only other one that showed any definite ability to decay radicles, apparently decreased sprouting in three out of the four experiments in which it was used. Seven of the eight units inoculated with these two lines gave lower germination than the uninoculated units. Irrespective of the magnitude of the differences, their uniformity of direction constitutes decided evidence that they are not accidental. In the experiments with numerous uninoculated units, and hence with relatively reliable basis for comparison, all the units inoculated with these two lines germinated less well than the uninoculated units. These differences expressed in terms of the probable error of a single uninoculated unit were 6.7, 2.7, 0.1, 4.3, 10.3, 2.2, and 5.3. Thus, the evidence is rather strong, though not conclusive because of the small number of tests, that the lines of *Botrytis cinerea* capable of decaying radicles were also capable of causing sprouting decrease.

TABLE 16.—*Relation between radicle decay and sprouting decrease caused by fungi in certain coniferous seeds during germination*

Inoculating fungus	Units with radicle decay		Units without radicle decay	
	Number	Percentage in which there was apparent sprouting decrease	Number	Percentage in which there was apparent sprouting decrease
Pythium aphanidermatum	3	100	0	.....
Pythiacystis citrophthora	2	100	0	.....
Pythium ultimum	40	82	0	.....
Phomopsis juniperovora	11	82	0	.....
Botrytis cinerea	9	78	8	50
Fusarium spp.	44	75	109	51
Phytophthora spp.	7	72	3	67
More virulent lines of Corticium vagum	35	71	0	.....
Less virulent lines of Corticium vagum	30	47	20	25
Botrytis spp. (small-sclerotium type)	0	.....	5	40
Other fungi	0	.....	16	44

Phomopsis juniperovora and *Phytophthora* sp. appeared to cause sprouting decrease in some units of *Pinus banksiana*, and in the one experiment with *P. resinosa* they decreased sprouting in all units inoculated with them. One unit of *P. banksiana* inoculated with *Cephalothecium roseum* showed no rotted radicles, but the germination was significantly lower than that in the average uninoculated

unit. In this case the germination differed from the mean of the uninoculated units by about five times the probable error of a single uninoculated unit. It seems, therefore, reasonable to believe that the addition of the Cephalothecium was somewhat effective, making the unit essentially different from the average uninoculated unit. However, despite the symmetrical distribution of sprouting percentages in the uninoculated units of all three large experiments, there was an unexplained lowering in the sprouting percentage of three of the uninoculated plates in the small experiment No. 120, which makes it appear that differences can not be interpreted strictly in terms of their probable errors, even in the larger experiments; and the data on this one plate, therefore, by no means establish the ability of Cephalothecium to cause seed decay. Further experimentation is necessary to substantiate this suggestion.

Most of the Fusarium and Corticium lines that were less virulent to young radicles, most of the *Botrytis cinerea* lines, the small sclerotium Botrytis lines, and the remaining fungi showed no appreciable apparent ability to decay unruptured seeds of *Pinus banksiana*. However, it is evident, from a consideration of the figures for *P. banksiana*, that with sprouting taking place as quickly as it did in these experiments (approximately two weeks after planting) the decay of unruptured seeds is a less important part of germination loss than the decay of radicles shortly after rupture of the seed coats. It is also obvious that the only fungi able to cause significant rotting of unruptured seeds were those able to attack radicles.

For *Pinus resinosa* the losses both before and after sprouting were, on the whole, about the same as for *P. banksiana*, but they were distributed differently, furnishing less support for correlation between ability to decay radicles soon after sprouting and that to cause sprouting decrease. Fusarium lines, for example, which caused no decay of radicles, apparently decreased the sprouting percentages in *P. resinosa*.

In the spruce experiment, in which there were only six uninoculated units, but these in excellent agreement with one another, there was distinct indication of seed decay by most of the fungi used, including many that had no effect at all on the radicles of the seed that sprouted. Because of the small number of units, especially of the uninoculated ones, great weight can not be attached to the results with this species.

The fact that the fungi able to attack radicles were also, in general, the ones able to decrease sprouting raises the question whether the seeds whose germination was prevented were really decayed while still truly dormant. It seems reasonable to believe, in view of the apparent relation between the ability of the various fungi to rot radicles and to prevent the germination of unruptured seed, that the seeds whose sprouting was prevented were attacked after the changes preceding germination had gone so far inside the seeds that they could no longer be considered truly dormant. It is, of course, also possible that some of the seed reported as unruptured may have really been ruptured but immediately killed by the penetration of hyphae of the parasite before the rupture of the seed coat had proceeded far enough to be detected by a rapid examination with a hand lens. Whatever the truth may be as to this, it is evident, from experiments in which the fungus enjoyed very favorable conditions for its

spread into the seed, that when germination is prompt such seed decay as occurs in the seed bed is likely to be of minor importance and caused chiefly by damping-off fungi. Where germination loss is heavy, most of the loss may usually be attributed to the infection of seeds that have already sprouted by damping-off fungi.

Attention has been called by some writers (*9, 12, 14, 17, 19, 31*) to the fact that old, injured, stored, or slowly germinating seeds may be decayed by saprophytic organisms; but, as others (*15, 16, 33*) have stated that coniferous seeds germinate after storage for several years in the forest floor, it is probable that really dormant seeds are not seriously decayed by fungi other than damping-off parasites. However, in an unpublished note Hartley reported that in some laboratory germination tests with *Pseudotsuga taxifolia*, Trichoderma, Mucor, and Penicillium, but no Pythium, Rhizoctonia, or Botrytis, were present in the seeds that failed to germinate.

SUMMARY

Experiments in which coniferous seeds were germinated in contact with agar cultures of fungi appear to warrant the following statement: Poor germination of pine, as a result of the work of damping-off parasites, is mainly due to the destruction of radicles after they have emerged from the seed coat but before the seedlings are large enough to break through the soil. Decay of unruptured seed apparently can be caused by these fungi, but it appears to be of less importance as a cause of germination loss.

Pythium ultimum, *P. aphanidermatum*, *Pythiacystis citrophthora*, *Phytophthora* spp., *Fusarium sporotrichioides*, *F. discolor sulphureum*, *F. arthrosporioides*, *Botrytis cinerea*, *Phomopsis juniperovora*, and some lines of *Corticium vagum* and *F. moniliforme* were able to decay radicles that had just emerged from the seed coats. Several other species of Fusarium attacked recently emerged radicles at times, but not frequently enough to establish either their parasitic or their nonparasitic ability.

The following fungi failed to attack radicles under the conditions of these experiments: *Cephalothecium roseum*, *Verticillium* sp., *Thielavia basicola*, *Pestalozzia funerea* (?), the small sclerotial *Botrytis* spp., and some of the *Corticium vagum* lines, *Fusarium radicicola*, *F. oxysporum*, *F. coeruleum*, *F. hyperoxysporum*, *F. avenaceum*, *F. orthoceras*(?), *F. ventricosum*, three lines of *F. acuminatum*, three lines belonging to the *discolor* section of Fusarium, *Pythium artotrogus*, *Mucor racemosus*, and an unidentified Phycomycete.

All the available lines of *Pythium ultimum* and some of the more virulent lines of *Corticium vagum* gave apparent evidence of attacking seeds that had not ruptured, so far as could be detected with a hand lens. *P. aphanidermatum*, *Pythiacystis citrophthora*, *Fusarium sporotrichioides*, and *Phomopsis juniperovora*, none of which have been isolated from damped-off coniferous seedlings under natural conditions, also appeared to attack unruptured seeds. Several of the other fungi gave less apparent evidence of this ability.

The results in the one experiment with *Pinus resinosa* agreed in general with those of *P. banksiana*, except that the seeds of *P. resinosa* showed reduced sprouting percentages with Fusarium lines and with certain other fungi that failed to attack radicles.

In a preliminary experiment *Picea engelmanni* appeared more susceptible to seed decay than either of the pines.

Pythium ultimum and the more virulent lines of *Corticium vagum*, in the experiments reported, caused more apparent germination loss than any tested species of Fusarium except *F. sporotrichioides*.

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GERMICIDAL EFFICIENCY OF SODIUM HYDROXIDE, SODIUM CARBONATE, AND TRISODIUM PHOSPHATE[1]

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INTRODUCTION

In this paper the writers present results which they obtained, for the most part, in making a comparative study of disinfectants that might be used by poultrymen, a fact which accounts for the choice of test organisms and various other details of the technic. Although, as the title implies, this work was done chiefly with the hydroxide, carbonate, and phosphate of sodium, the paper also reports the results of tests with various commonly used disinfectants.

EXPERIMENTAL PROCEDURE

The solutions of sodium hydroxide used for the bacteriological tests were made from standardized carbonate-free stock solutions already prepared for chemical work; the solutions of sodium carbonate were made from the anhydrous chemically pure salt; and the solutions of trisodium phosphate were made from the crystalline chemically pure salt containing 12 molecules of water, concentrations being given in the tables on a basis of trisodium phosphate without regard to water. The calcium hydroxide used in some of the experiments was usually prepared by hydration of United States Pharmacopoeia calcium oxide, but in some instances a high-grade commercial sample of hydrate of lime was employed.

Bacteriological tests were made by the Rideal-Walker technic, modified as described in a previous paper published in this journal,[2] and also by tests made at various medication temperatures instead of at a uniform temperature of 25° C.

RESULTS OF EXPERIMENTS

The results of bacteriological tests with sodium hydroxide against *Eberthella typhi*[3] (*Bacillus typhosus*), *Staphylococcus aureus*, *Salmonella pullorum*, *Pasteurella avicida*, *Salmonella gallinarum*, and two strains of hemolytic streptococci are shown in Table 1. In general, the results indicate that sodium hydroxide is an efficient germicide against these organisms even in the presence of organic matter in the form of skim milk.

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[1] Received for publication Sept. 25, 1930; issued January, 1931.

[2] SCHAFFER, J. M., and TILLEY, F. W. GERMICIDAL EFFICIENCY OF SOAPS AND OF MIXTURES OF SOAPS WITH SODIUM HYDROXIDE OR WITH PHENOLS. Jour. Agr. Research 41: 737–747. 1930.

[3] The bacteriological nomenclature used in this paper is that of the following publication: BERGEY, D. H. BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY; A KEY FOR THE IDENTIFICATION OF ORGANISMS OF THE CLASS SCHIZOMYCETES * * * Ed. 3, 589 p., Baltimore. 1930.

TABLE 1.—*Germicidal efficiency of sodium hydroxide against Eberthella typhi, Staphylococcus aureus, and other test organisms* [a]

Disinfectant	Dilution	Organic matter	Growth [b] after indicated period of exposure (minutes)						Test organism
			2½	5	7½	10	12½	15	
Phenol	1-100	None	+	+	−	−	−	−	E. typhi No. 1.
NaOH	1-1,400	do	+	+	−	−	−	−	Do.
Do	1-1,600	do	+	+	+	−	−	−	Do.
Do	1-1,800	do	+	+	+	+	−	−	Do.
Do	1-300	Skim milk, 50 per cent.	−	−	−	−	−	−	Do.
Do	1-400	do	+	+	−	−	−	−	Do.
Do	1-500	do	+	+	+	+	+	+	Do.
Phenol	1-95	None	+	+	+	−	−	−	E. typhi No. 2.
NaOH	1-1,200	do	+	+	+	−	−	−	Do.
Do	1-1,600	do	+	+	+	+	+	+	Do.
Phenol	1-70	do	+	+	−	−	−	−	Staph. aureus.
NaOH	1-80	do	+	+	−	−	−	−	Do.
Do	1-100	do	+	+	+	−	−	−	Do.
Do	1-120	do	+	+	+	+	−	−	Do.
Do	1-50	Skim milk, 50 per cent.	+	+	+	−	−	−	Do.
Do	1-60	do	+	+	+	+	−	−	Do.
Do	1-70	do	+	+	+	+	+	+	Do.
Phenol	1-90	None	+	+	+	−	−	−	Salmonella pullorum.
NaOH	1-1,200	do	+	+	+	−	−	−	Do.
Do	1-1,600	do	+	+	+	+	+	+	Do.
Do	1-300	Skim milk, 50 per cent.	+	+	+	−	−	−	Do.
Do	1-400	do	+	+	+	+	+	+	Do.
Phenol	1-160	None	+	+	−	−	−	−	Pasteurella avicida.
NaOH	1-1,600	do	+	−	−	−	−	−	Do.
Do	1-2,000	do	+	+	+	−	−	−	Do.
Do	1-400	Skim milk, 50 per cent.	+	−	−	−	−	−	Do.
Do	1-500	do	+	+	+	+	−	−	Do.
Phenol	1-100	None	+	+	+	+	−	−	Salmonella gallinarum.
NaOH	1-1,200	do	+	+	−	−	−	−	Do.
Do	1-1,600	do	+	+	+	+	+	+	Do.
Do	1-200	Skim milk, 50 per cent.	+	+	−	−	−	−	Do.
Do	1-400	do	+	+	+	+	+	+	Do.
NaOH	1-100	None	+	+	−	−	−	−	Streptococcus No. 1.
Do	1-150	do	+	+	+	+	+	+	Do.
Do	1-600	do	+	+	+	−	−	−	Streptococcus No. 2.
Do	1-800	do	+	+	+	+	+	+	Do.

[a] Experiments conducted at 25° C. [b] + indicates growth; −, no growth.

TABLE 2.—*Germicidal efficiency of various disinfectants against Salmonella pullorum* [a]

Disinfectant	Dilution	Organic matter	Growth [b] after indicated period of exposure (minutes)					
			2½	5	7½	10	12½	15
Phenol	1-90	None	+	+	−	−	−	−
Formalin	1-20	do	−	−	−	−	−	−
Do	1-40	do	+	+	+	−	−	−
Do	1-60	do	+	+	+	+	+	+
Do	1-30	Skim milk, 50 per cent	+	+	+	−	−	−
Do	1-40	do	+	+	+	+	+	+
Cresol, U. S. P.	1-240	None	+	−	−	−	−	−
Do	1-280	do	+	+	+	+	−	−
Do	1-260	Skim milk, 50 per cent	+	+	+	−	−	−
Do	1-220	do	+	+	+	+	+	+
Liquor cresolis compositus, U. S. P.	1-160	None	+	−	−	−	−	−
Do	1-200	do	+	+	+	−	−	−
Do	1-240	do	+	+	+	+	+	−
Do	1-120	Skim milk, 50 per cent	+	+	−	−	−	−
Do	1-140	do	+	+	+	+	+	+
Lime water		None	−	−	−	−	−	−
Milk of lime		Skim milk, 50 per cent	+	−	−	−	−	−
NaOH	1-1,600	None	+	+	+	−	−	−
Do	1-400	Skim milk, 50 per cent	+	+	+	−	−	−

[a] Experiments conducted at 25° C. [b] + indicates growth; −, no growth.

Experiments were next made with the same technic to ascertain the efficiency of various other disinfectants as compared with that of sodium hydroxide. The results of these comparative tests are shown in Table 2. Then in order to test all the disinfectants under conditions more closely approximating those met in practice, experiments were made with organic matter in the form of chicken feces, sterilized in the autoclave to avoid complications from bacteria already present, 1 gram of feces to 5 c. c. of disinfectant being used. The results, shown in Table 3, indicate that a 1 per cent solution of sodium hydroxide is effective, in exposures of 5 minutes or more, against all the three test organisms except *Staphylococcus aureus*, which requires a 3 per cent solution.

TABLE 3.—*Germicidal efficiency against various organisms [a] of sodium hydroxide and other disinfectants in the presence of chicken feces [b]*

Disinfectant	Dilution	Growth [c] after indicated period of exposure (minutes)			Test organism
		5	10	15	
Phenol	1- 80	+	−	−	Salmonella pullorum.
Cresol U. S. P.	1-160	−	−	−	Do.
Do	1-200	+	+	−	Do.
Liquor cresolis compositus U. S. P.	1-100	−	−	−	Do.
Do	1-150	+	+	+	Do.
Formalin	1- 20	−	−	−	Do.
Do	1- 30	+	−	−	Do.
Milk of lime		−	−	−	Do.
NaOH	1-100	−	−	−	Do.
Do	1-200	+	+	+	Do.
Do	1-200	−	−	−	Pasteurella avicida.
Do	1-300	+	−	−	Do.
Do	1-400	+	+	+	Do.
Do	1-200	−	−	−	Salmonella gallinarum.
Do	1-300	+	+	+	Do.
Do	1- 33	−	−	−	Staphylococcus aureus.
Do	1- 50	+	−	−	Do.
Do	1-100	+	+	+	Do.

[a] Experiments conducted at 25° C.
[b] 1 gm. feces to 5 c. c. disinfectant.
[c] + Indicates growth; −, no growth.

A study was then made of the germicidal efficiency of sodium carbonate and of mixtures containing sodium carbonate and sodium hydroxide. The results obtained are shown in Tables 4, 5, 6, and 7. Table 5 also shows results obtained with mixtures containing sodium hydroxide and sodium chloride.

TABLE 4.—*Germicidal efficiency of sodium carbonate against Salmonella pullorum*

EXPERIMENT 1

Na_2CO_3 dilution	Organic matter	Growth [a] after indicated period of exposure (minutes)						Medication temperature
		2½	5	7½	10	12½	15	
								° C.
1 to 5	None	+	+	+	+	+	+	25
1 to 10	do	+	+	+	−	−	−	25
1 to 20	do	+	+	−	−	−	−	25
1 to 40	do	+	+	−	−	−	−	25
1 to 80	do	+	+	+	+	−	−	25

EXPERIMENT 2

		15	30	45	60	90	120	
								° C.
1 to 100	None	+	−	−	−	−	−	(b)
1 to 200	do	+	+	+	+	+	[c] −	(b)
1 to 300	do	+	+	+	+	+	+	(b)
1 to 400	do	+	−	−	−	−	−	40
1 to 500	do	+	+	−	−	−	−	40
1 to 600	do	+	+	+	−	−	−	40
1 to 800	do	−	−	−	−	−	−	50
1 to 1,200	do	+	+	−	−	−	−	50
1 to 1,600	do	+	+	+	−	−	−	50
1 to 2,000	do	+	+	+	+	+	−	50
[d]	Distilled water	+	+	+	+	+	+	50

EXPERIMENT 3

		2½	5	7½	10	12½	15	
								° C.
1 to 40	Skim milk, 50 per cent	+	−	−	−	−	−	40
1 to 60	do	+	+	+	−	−	−	40
1 to 80	do	+	+	+	+	−	−	40
1 to 100	do	+	+	+	+	+	−	40
1 to 80	do	−	−	−	−	−	−	50
1 to 120	do	+	−	−	−	−	−	50
1 to 160	do	+	−	−	−	−	−	50
1 to 200	do	+	+	−	−	−	−	50
1 to 240	do	+	+	−	−	−	−	50
1 to 280	do	+	+	+	+	+	+	50

[a] + indicates growth; −, no growth.
[b] Room temperature, between 22° and 32° C.
[c] Growth after 5 hours.
[d] No Na_2CO_3 used.

TABLE 5.—*Germicidal efficiency of sodium hydroxide and sodium carbonate or sodium chloride against Salmonella pullorum*

Na_2CO_3 or NaCl	Dilution	NaOH dilution	Growth [a] after indicated period of exposure (minutes)										Medication temperature
			5	10	15	30	45	60	90	120	150	300	
													° C.
[b]		1-1,000	−	−	−	−	−	−					[c]
[b]		1-1,500	+	+	+	−	−	−					[c]
[b]		1-2,000	+	+	+	+	+	+	+	−			[c]
[b]		1-2,500	+	+	+	+	+	+	+	+	+	+	[c]
Na_2CO_3	1-200	1-4,000	+	+	+	−	−	−					[c]
Do	1-200	1-8,000	+	+	+	+	+	−					[c]
Do	1-200	1-12,000	+	+	+	+	+	+	+	−			[c]
Do	1-600	1-1,500	+	+	−	−	−	−					[c]
Do	1-600	1-2,000	+	+	+	−	−	−					[c]
Do	1-600	1-2,500	+	+	+	+	+	−					[c]
Do	1-200	1-1,500	+	+	−	−	−	−					[c]
Do	1-200	1-2,000	+	+	+	+	−	−					[c]
NaCl	1-200	1-2,500	+	+	+	+	+	+	+	+	−		[c]
[b]		1-2,000	+	+	+	−	−	−					40
[b]		1-3,000	+	+	+	+	+	+	+	+			40
Na_2CO_3	1-800	1-2,000	+	+	−	−	−	−					40
Do	1-800	1-4,000	+	+	+	+	−	−					40
Do	1-800	1-6,000	+	+	+	+	+	+					40
Do	1-200	1-2,000	+	+	+	−	−	−					40
NaCl	1-200	1-3,000	+	+	+	+	+	+	+	+			40
[b]		1-2,000	−	−	−	−	−	−					50
[b]		1-3,000	+	+	+	−	−	−					50
[b]		1-4,000	+	+	+	+	+	−					50
Na_2CO_3	1-2,000	1-4,000	+	−	−	−	−	−					50
NaCl	1-200	1-3,000	+	+	−	−	−	−					50
Do	1-200	1-4,000	+	+	+	+	+	−					50

[a] + indicates growth; −, no growth.
[b] No Na_2CO_3 nor NaCl used.
[c] Room temperature, between 22° and 32° C.

TABLE 6.—*Germicidal efficiency of sodium hydroxide and sodium carbonate against Salmonella pullorum with organic matter present*

Na_2CO_3 dilution	NaOH dilution	Organic matter	Growth [a] after indicated period of exposure (minutes)						Medication temperature
			5	10	15	30	45	60	
									° C.
1 to 50	1-400	Skim milk, 50 per cent	−	−	−	−	−	−	[b]
Do	1-600	do	+	+	−	−	−	−	[b]
Do	1-800	do	+	+	−	−	−	−	[b]
1 to 100	1-400	do	−	−	−	−	−	−	[b]
Do	1-600	do	+	+	−	−	−	−	[b]
Do	1-800	do	+	+	+	−	−	−	[b]
1 to 200	1-400	do	−	−	−	−	−	−	[b]
Do	1-600	do	+	+	+	−	−	−	[b]
Do	1-800	do	+	+	+	+	+	−	[b]
Do	1-500	do	+	−	−	−	−	−	40
Do	1-1,000	do	+	+	+	−	−	−	40
Do	1-2,000	do	+	+	+	−	−	−	40
1 to 400	1-400	do	+	−	−	−	−	−	40
Do	1-600	do	+	+	−	−	−	−	40
Do	1-800	do	+	+	+	−	−	−	40
1 to 800	1-1,000	do	−	−	−	−	−	−	50
Do	1-1,500	do	+	+	+	−	−	−	50
Do	1-2,000	do	+	+	+	+	−	−	50
Do	1-3,000	do	+	+	+	+	+	−	50

[a] + indicates growth; −, no growth.
[b] Room temperature, between 22° and 32° C.

TABLE 7.—*Germicidal efficiency against Staphylococcus aureus of sodium hydroxide and sodium carbonate without and with organic matter present*

Na_2CO_3 dilution	NaOH dilution	Organic matter	Growth [a] after indicated period of exposure (minutes)						Medication temperature
			5	10	15	30	45	60	
									°C.
1 to 50	[b]	None	+	+	+	+	+	[c]+	25
[d]	1-100	do	+	−	−	−	−	−	25
[d]	1-150	do	+	+	+	−	−	−	25
1 to 100	1-100	do	+	−	−	−	−	−	25
Do	1-200	do	+	+	+	−	−	−	25
Do	1-400	do	+	+	+	+	+	−	25
1 to 5	[b]	do	+	+	+	+	−	−	40
1 to 40	[b]	do	+	+	+	−	−	−	40
[d]	1-400	do	+	+	+	+	−	−	40
[d]	1-800	do	+	+	−	−	−	−	40
1 to 50	1-400	do	+	+	+	+	+	−	40
Do	1-800	do	+	−	−	−	−	−	40
Do	1-1, 600	do	+	+	−	−	−	−	40
1 to 100	1-400	do	+	+	+	−	−	−	40
Do	1-800	do	+	−	−	−	−	−	40
Do	1-1, 600	do	+	+	−	−	−	−	40
1 to 200	1-400	do	+	+	+	−	−	−	40
Do	1-800	do	+	−	−	−	−	−	40
Do	1-1, 200	do	+	+	−	−	−	−	40
Do	1-1, 600	do	+	+	+	−	−	−	40
1 to 5	[b]	do	+	+	+	+	+	−	40
1 to 40	[b]	do	+	−	−	−	−	−	50
[d]	1-400	do	+	−	−	−	−	−	50
[d]	1-800	do	+	−	−	−	−	−	50
[d]	1-1, 600	do	+	+	+	−	−	−	50
1 to 200	1-1, 600	do	+	+	+	+	+	−	50
Do	1-2, 000	do	+	−	−	−	−	−	50
Do	1-2, 400	do	+	+	−	−	−	−	50
1 to 400	1-1, 600	do	+	+	+	−	−	−	50
Do	1-2, 000	do	+	−	−	−	−	−	50
Do	1-2, 400	do	+	+	−	−	−	−	50
1 to 800	1-800	do	+	+	+	−	−	−	50
Do	1-1, 200	do	+	−	−	−	−	−	50
Do	1-1, 600	do	+	+	+	−	−	−	50
Do	1-2, 000	do	+	+	+	−	−	−	50
1 to 10	[b]	Skim milk, 50 per cent	+	+	+	+	+	+	50
[d]	1-100	do	+	+	+	+	+	[e]+	40
1 to 50	1-50	do	+	+	+	+	−	−	40
Do	1-100	do	−	−	−	−	−	[f]−	40
Do	1-200	do	+	−	−	−	−	−	40
Do	1-400	do	+	+	+	−	−	−	40
1 to 100	1-50	do	+	+	+	+	−	−	40
Do	1-100	do	−	−	−	−	−	[f]−	40
Do	1-200	do	+	+	−	−	−	−	40
Do	1-400	do	+	+	+	−	−	−	40
1 to 40	[b]	Skim milk, 10 per cent	+	+	+	+	+	−	40
[d]	1-200	do	+	+	+	+	+	−	40
1 to 100	1-200	do	+	+	−	−	−	−	40
Do	1-400	do	+	+	−	−	−	−	40
Do	1-600	do	+	+	−	−	−	−	40
1 to 40	[b]	Skim milk, 50 per cent	+	+	+	−	−	−	40
[d]	1-200	do	+	+	−	−	−	−	50
1 to 100	1-100	do	+	−	−	−	−	−	50
Do	1-200	do	−	−	−	−	−	[f]−	50
Do	1-400	do	+	−	−	−	−	−	50
Do	1-800	do	+	+	−	−	−	−	50
			+	+	+	−	−	−	50

[a] + indicates growth; −, no growth.
[b] No NaOH used.
[c] Growth after 24 hours' exposure.
[d] No Na_2CO_3 used.
[e] Growth after 1½ hours' exposure; no growth after 2 hours.
[f] No growth after 2½ minutes' exposure.

In general, these results indicate that sodium carbonate itself has comparatively little germicidal efficiency at temperatures approximating 25° C. but that it is effective at higher temperatures, even in the presence of organic matter. The addition of sodium carbonate to solutions of sodium hydroxide greatly increases the efficiency of such solutions, and the efficiency of the mixtures is still further increased by heat. Tables 6 and 7 show the quantities of sodium carbonate in proportion to sodium hydroxide that are of value, and also that if more of the carbonate is used comparatively little is added to the germicidal efficiency of the solutions. The addition of sodium chloride to solutions of sodium hydroxide produces a comparatively small increase in efficiency at ordinary temperatures with practically no increase at higher temperatures.

Results of tests with mixtures containing sodium carbonate and various soaps, shown in Table 8, indicate that the carbonate considerably increases the germicidal efficiency of soap solutions.

TABLE 8.—*Germicidal efficiency of mixtures containing sodium carbonate and soap against Salmonella pullorum* [a]

Na_2CO_3 dilution	Soap	Soap dilution	Growth [b] after indicated period of exposure (minutes)					
			5	10	15	20	25	30
(c)	Coconut oil	1-20	−	−	−	−	−	−
(c)	do	1-30	+	+	+	−	−	−
(c)	do	1-40	+	+	+	+	+	+
(c)	Castor oil	1-10	+	+	+	+	+	+
(c)	Linseed oil	1-10	+	+	+	+	+	−
1 to 50	None		+	−	−	−	−	−
1 to 100	do		+	+	+	+	+	+
1 to 200	Castor oil	1-500	+	−	−	−	−	−
Do	do	1-1,000	+	+	−	−	−	−
Do	do	1-1,500	+	+	+	−	−	−
Do	do	1-1,200	+	+	+	+	−	−
Do	do	1-2,000	+	+	+	+	+	−
Do	Coconut oil	1-500	−	−	−	−	−	−
Do	do	1-1,000	+	−	−	−	−	−
Do	do	1-1,500	+	+	+	−	−	−
Do	do	1-2,000	+	+	+	+	−	−
Do	do	1-2,500	+	+	+	+	+	−
Do	Linseed oil	1-25	+	+	−	−	−	−
Do	do	1-50	+	+	−	−	−	−
Do	do	1-100	+	+	+	−	−	−
Do	do	1-200	+	+	+	+	−	−
Do	do	1-400	+	+	+	+	−	−
Do	do	1-800	+	+	+	+	−	−
Do	do	1-1,200	+	+	+	+	−	−
Do	do	1-1,600	+	+	+	+	−	−
Do	do	1-2,000	+	+	+	+	−	−
Do	do	1-2,400	+	+	+	+	−	−

[a] Experiments conducted at 25° C.
[b] + indicates growth; −, no growth.
[c] No Na_2CO_3 used.

The results of experiments with trisodium phosphate and mixtures containing trisodium phosphate and sodium hydroxide are shown in Table 9, and results obtained with mixtures containing trisodium phosphate and soaps are shown in Table 10. These results indicate that trisodium phosphate has greater germicidal efficiency than sodium carbonate and that it is also more effective in increasing the germicidal efficiency of sodium hydroxide solutions and of soap solutions except in some instances when *Staphylococcus aureus* was used.

TABLE 9.—*Germicidal efficiency against Salmonella pullorum and Staphylococcus aureus of trisodium phosphate and its mixtures with sodium hydroxide without and with organic matter present*

Na_3PO_4 dilution	NaOH dilution	Organic matter	Growth [a] after indicated period of exposure (minutes)						Medication temperature	Test organism
			5	10	15	30	45	60		
									° C.	
1 to 200	[b]	None	−	−	−	−	−	−	[c]	S. pullorum.
1 to 400	[b]	do	+	+	+	−	−	−	[c]	Do.
1 to 600	[b]	do	+	+	+	+	+	+	[c]	Do.
[d]	1–1, 500	do	+	+	+	−	−	−	[c]	Do.
[d]	1–2, 000	do	+	+	+	+	+	+	[c]	Do.
1 to 600	1–2, 000	do	+	−	−	−	−	−	[c]	Do.
Do	1–3, 000	do	+	+	−	−	−	−	[c]	Do.
Do	1–4, 000	do	+	+	+	−	−	−	[c]	Do.
1 to 50	[b]	do	+	+	+	+	+	+	[c]	Staph. aureus.
[d]	1–100	do	+	+	−	−	−	−	[c]	Do.
[d]	1–200	do	+	+	+	+	−	−	[c]	Do.
1 to 200	1–200	do	+	+	+	−	−	−	[c]	Do.
Do	1–400	do	+	+	+	+	+	−	[c]	Do.
1 to 50	[b]	do	+	−	−	−	−	−	40	Do.
1 to 100	[b]	do	+	+	+	−	−	−	40	Do.
[d]	1–400	do	+	+	+	−	−	−	40	Do.
1 to 200	1–400	do	+	−	−	−	−	−	40	Do.
Do	1–800	do	+	+	+	+	−	−	40	Do.
Do	1–1, 200	do	+	+	+	+	−	−	40	Do.
Do	[b]	do	+	−	−	−	−	−	50	Do.
1 to 400	[b]	do	+	+	+	+	+	−	50	Do.
[d]	1–600	do	+	+	−	−	−	−	50	Do.
[d]	1–800	do	+	+	+	−	−	−	50	Do.
1 to 400	1–1, 600	do	+	−	−	−	−	−	50	Do.
Do	1–2, 000	do	+	+	+	−	−	−	50	Do.
1 to 800	1–800	do	+	−	−	−	−	−	50	Do.
Do	1–1, 200	do	+	+	−	−	−	−	50	Do.
1 to 100	[b]	Skim milk, 50 per cent	+	−	−	−	−	−	[c]	S. pullorum.
1 to 200	[b]	do	+	+	+	+	+	+	[c]	Do.
[d]	1–400	do	+	−	−	−	−	−	[c]	Do.
[d]	1–500	do	+	+	+	−	−	−	[c]	Do.
1 to 200	1–800	do	−	−	−	−	−	−	[c]	Do.
Do	1–1, 200	do	+	+	−	−	−	−	[c]	Do.
Do	1–1, 600	do	+	+	+	+	−	−	[c]	Do.
1 to 25	[b]	do	+	+	+	−	−	−	40	Staph. aureus.
1 to 50	[b]	do	+	+	+	+	−	−	40	Do.
[d]	1–100	do	+	+	−	−	−	−	40	Do.
[d]	1–200	do	+	+	+	+	−	−	40	Do.
1 to 50	1–200	do	+	+	−	−	−	−	40	Do.
Do	1–400	do	+	+	+	+	−	−	40	Do.
1 to 100	1–200	do	+	+	+	−	−	−	40	Do.
Do	1–400	do	+	+	+	+	−	−	40	Do.
1 to 50	[b]	Skim milk, 10 per cent	+	+	+	−	−	−	40	Do.
1 to 100	[b]	do	+	+	+	−	−	−	40	Do.
[d]	1–200	do	+	+	−	−	−	−	40	Do.
[d]	1–400	do	+	+	+	−	−	−	40	Do.
1 to 50	1–800	do	+	−	−	−	−	−	40	Do.
Do	1–1, 200	do	+	+	−	−	−	−	40	Do.
1 to 100	1–400	do	+	−	−	−	−	−	40	Do.
Do	1–800	do	+	+	−	−	−	−	40	Do.
1 to 50	[b]	Skim milk, 50 per cent	+	+	+	−	−	−	50	Do.
1 to 100	[b]	do	+	+	+	+	−	−	50	Do.
[d]	1–200	do	+	+	+	+	−	−	50	Do.
[d]	1–400	do	+	+	+	+	+	−	50	Do.
1 to 50	1–800	do	+	+	−	−	−	−	50	Do.
Do	1–1, 200	do	+	+	+	−	−	−	50	Do.
1 to 100	1–400	do	+	+	+	−	−	−	50	Do.
Do	1–800	do	+	+	+	+	−	−	50	Do.

[a] + indicates growth; −, no growth.
[b] No NaOH used.
[c] Room temperatures between 22° and 32° C.
[d] No Na_3PO_4 used.

TABLE 10.—*Germicidal efficiency of mixtures containing trisodium phosphate and soap against Salmonella pullorum and Staphylococcus aureus* [a]

Na_3PO_4 dilution	Soap	Soap dilution	Growth [b] after indicated period of exposure (minutes)						Test organism
			5	10	15	30	45	60	
1 to 600	Castor oil	1–1,000	+	−	−	−	−	−	S. pullorum.
Do	do	1–2,000	+	+	+	−	−	−	Do.
Do	do	1–3,000	+	+	+	+	−	−	Do.
Do	do	1–4,000	+	+	+	+	+	−	Do.
Do	Coconut oil	1–1,000	+	−	−	−	−	−	Do.
Do	do	1–2,000	+	+	+	−	−	−	Do.
Do	do	1–3,000	+	+	+	+	−	−	Do.
Do	do	1–4,000	+	+	+	+	+	−	Do.
[c]	do	1–5	+	+	+	+	+	+	Staph. aureus.
1 to 100	do	1–20	+	+	+	−	−	−	Do.
Do	do	1–40	+	+	+	−	−	−	Do.
Do	do	1–60	+	+	+	−	−	−	Do.
Do	do	1–80	+	+	+	−	−	−	Do.
Do	do	1–100	+	+	+	−	−	−	Do.
1 to 200	do	1–60	+	+	+	−	−	−	Do.
Do	do	1–80	+	+	+	−	−	−	Do.
Do	do	1–100	+	+	+	+	+	+	Do.

[a] Experiments conducted at room temperature (between 22° and 32° C.).
[b] + indicates growth; −, no growth.
[c] No Na_3PO_4 used.

The results of experiments with sodium hydroxide and calcium hydroxide against *Staphylococcus aureus*, shown in Table 11, indicate that calcium hydroxide increases the germicidal efficiency of sodium hydroxide against that test organism.

TABLE 11.—*Germicidal efficiency of sodium hydroxide and calcium hydroxide against Staphylococcus aureus* [a]

$Ca(OH)_2$ dilution	NaOH dilution	Organic matter	Growth [b] after indicated period of exposure (minutes)								
			5	10	15	20	30	45	60	90	120
[c]	1 to 100	None	+	−	−	−	−	−	−	.....	.....
[c]	1 to 200	do	+	+	+	−	−	−	−	.....	.....
1 to 100	[d]	do	+	+	+	+	+	−	−	.....	.....
1 to 20	[d]	do	+	+	+	+	+	−	−	.....	.....
Do	1 to 100	do	+	−	−	−	−	−	−	.....	.....
Do	1 to 200	do	+	+	−	−	−	−	−	.....	.....
Do	1 to 400	do	+	+	+	−	−	−	−	.....	.....
[c]	1 to 50	Chicken feces [e]	+	+	−	−	−	−	−	.....	.....
[c]	1 to 100	do	+	+	+	+	+	+	+	+	+
1 to 100	[d]	do	+	+	+	+	+	+	+	−	−
1 to 20	[d]	do	+	+	+	+	+	+	−	−	−
1 to 100	1 to 100	do	+	+	+	+	−	−	−	.....	.....
1 to 20	1 to 50	do	+	−	−	−	−	−	−	.....	.....
Do	1 to 100	do	+	+	+	−	−	−	−	.....	.....
Do	1 to 200	do	+	+	+	+	−	−	−	.....	.....
Do	1 to 400	do	+	+	+	+	−	−	−	.....	.....
Do	1 to 800	do	+	+	+	+	+	+	+	−	−

[a] Experiments conducted at room temperature (between 22° and 32° C.).
[b] + indicates growth; −, no growth.
[c] No $Ca(OH)_2$ used.
[d] No NaOH used.
[e] 1 gm. chicken feces to 5 c. c. disinfectant.

Table 12 presents the results of experiments with sodium hydroxide and with mixtures of sodium hydroxide and calcium hydroxide against spores of *Bacillus anthracis*. In experiment 1, one-half of a cubic centimeter of a spore suspension containing approximately 1,000,000 spores per cubic centimeter was added to 5 c. c. of disinfectant; whereas in experiment 2, 5 c. c. of disinfectant was added to a mixture containing 4 c. c. of defibrinated horse blood and 1 c. c. of the above-mentioned spore suspension. It is obvious, therefore, that in experiment 2 the initial concentrations of sodium hydroxide and calcium hydroxide were necessarily just twice as great as the concentrations shown in the table. Results obtained with mercuric chloride are shown only for comparison.

TABLE 12.—*Germicidal efficiency of sodium hydroxide and of sodium hydroxide with calcium hydroxide against spores of Bacillus anthracis*

EXPERIMENT 1[a]

$Ca(OH)_2$	NaOH	Growth[b] after indicated period of exposure (minutes)					
		15	30	45	60	90	120
Per cent	*Per cent*						
0	10	+	−	−	−	−	−
0	8	+	+	−	−	−	−
0	6	+	+	−	−	−	−
0	5	+	+	+	−	−	−
0	4	+	+	+	+	−	−
5	5	+	+	+	+	−	−
10	5	+	+	+	+	−	−
5	4	+	+	+	+	−	−
10	4	+	+	+	+	−	−

EXPERIMENT 2[a]

Blood	$Ca(OH)_2$	NaOH	Growth[b] after indicated period of exposure (hours)					
			1	1½	2	3	4	5
Per cent	*Per cent*	*Per cent*						
40	0	5.0	+	−	−	−	−	−
40	0	2.5	+	+	+	−	−	−
40	2.5	2.5	+	+	+	+	−	−
40	5.0	2.5	+	+	+	+	−	−
40	(c)	(d)	+	+	+	+	+	+

[a] Experiment conducted at room temperature, approximately 32° C.
[b] + indicates growth; −, no growth.
[c] $HgCl_2$ used.
[d] Dilution of 1-2,000.

It is well known that sodium hydroxide in solution when exposed to the air is converted to sodium carbonate. In view of this fact, experiments were conducted to ascertain the possible effect of such conversion upon germicidal efficiency. The results of the bacteriological tests are shown in Table 13, and the results of the chemical tests in Table 14.

TABLE 13.—*Germicidal efficiency of sodium hydroxide and of sodium hydroxide and calcium hydroxide against various organisms in open and closed containers*

EXPERIMENT 1 [a]

Organic matter (blood)	$Ca(OH)_2$	NaOH	Growth [b] after indicated period of exposure (hours)						Container	Test organism
			1	1½	2	3	4	5		
Per cent	*Per cent*	*Per cent*								
40	0	5.0	+	−	−	−	−	−	Closed	Spores of Bacillus anthracis.
40	0	5.0	+	−	−	−	−	−	Open	Do.
40	0	2.5	+	+	+	−	−	−	Closed	Do.
40	0	2.5	+	+	+	−	−	−	Open	Do.
40	2.5	2.5	+	+	+	+	−	−	Closed	Do.
40	2.5	2.5	+	+	+	−	−	−	Open	Do.

EXPERIMENT 2 [a]

Organic matter (skim milk)	$Ca(OH)_2$	NaOH dilution	Growth [b] after indicated period of exposure (minutes)						Container	Test organism
			5	10	15	20	25	30		
Per cent	*Per cent*									
0	0	1–150	+	+	−	−	−	−	Closed	Staphylococcus aureus.
0	0	1–150	+	+	−	−	−	−	Open	Do.
50	0	1–120	+	+	+	+	−	−	Closed	Do.
50	0	1–120	+	+	+	−	−	−	Open	Do.
0	0	1–1,200	+	−	−	−	−	−	do	Salmonella pullorum.
0	0	1–1,500	+	−	−	−	−	−	Closed	Do.
0	0	1–1,500	+	+	+	+	+	+	Open	Do.
50	0	1–400	−	−	−	−	−	−	Closed	Do.
50	0	1–400	−	−	−	−	−	−	Open	Do.
50	0	1–500	+	+	+	−	−	−	Closed	Do.
50	0	1–500	+	+	+	+	+	+	Open	Do.

[a] Experiment conducted at room temperature, approximately 32° C.
[b] + indicates growth; −, no growth.

TABLE 14.—*Conversion of sodium hydroxide to sodium carbonate on exposure to air*

EXPERIMENT 1

Initial NaOH present	Period of exposure	Final NaOH present	Na_2CO_3 formed	Unchanged NaOH
Per cent	*Minutes*	*Per cent*	*Per cent*	*Per cent*
1	15	0.67	0.69	60
1	30	.33	1.24	26
2	15	1.56	.86	71
2	30	1.44	1.21	61

EXPERIMENT 2

1	1	.....	.....	80–90
1	5	.....	.....	40
1	10	.....	.....	10
2	1	.....	.....	90–95
2	5	.....	.....	43
2	15	.....	.....	0

EXPERIMENT 3

	Hours			
1	18	.....	.....	64
1	24	.....	.....	53
2	18	.....	.....	73
2	24	.....	.....	64
2+10 per cent $Ca(OH)_2$	48	.....	.....	100

In experiment 2 (Table 13) the open containers were uncovered Petri dishes, whereas in experiment 1 they were sterile glass plates on which the material to be disinfected was spread out. In both experiments the "closed" containers were test tubes closed with cotton plugs.

Other experiments were conducted with sterile strips of filter paper as test objects. These strips were soaked in a culture of *Staphylococcus aureus* and then transferred to open Petri dishes and allowed to dry for about 10 minutes. A sodium-hydroxide solution was then poured into the dishes, and the filter-paper strips were thoroughly wet with the solution. Half of the strips were spread out in dry, open Petri dishes, whereas the others remained in contact with the sodium-hydroxide solution in closed dishes. The results obtained were similar to those shown for *S. aureus* in experiment 2 of Table 13.

In experiment 1 of Table 14, weighed filter papers were soaked in sodium-hydroxide solution, weighed again to determine the amount of solution taken up, and then exposed to the air in shallow dishes. At the end of each exposure period one of the filter papers was weighed again, and then transferred to a beaker containing distilled water, and thoroughly washed. The resulting solution was immediately titrated with suitable indicators to determine the amounts of sodium hydroxide and sodium carbonate present. The percentage figures in the fifth column were corrected to allow for evaporation. The differences between these figures and those shown in the third column indicate the amount of relative increase in concentration of sodium hydroxide due to evaporation.

In experiment 2 of Table 14, solutions of sodium hydroxide were sprayed in a fine mist across 2 feet of space upon filter papers fastened to the wall. At the end of the exposure periods the papers were washed off in distilled water, and the resulting solutions were titrated for sodium hydroxide and sodium carbonate.

In experiment 3 of Table 14, solutions of sodium hydroxide, with and without lime, were exposed to the air in shallow dishes. The results indicate that the lime acted to maintain the concentration of the sodium hydroxide. In tightly covered containers there was no appreciable change of hydroxide to carbonate even after a considerable length of time.

DISCUSSION

The results shown in the preceding tables indicate that sodium hydroxide, by itself or in mixtures with sodium carbonate, trisodium phosphate or calcium hydroxide, is an efficient disinfectant for use against many of the spore-bearing bacteria and also against the spores of *Bacillus anthracis*. The work of the American commission to study foot-and-mouth disease [4] has shown that sodium hydroxide is very effective against the virus of that disease. According to Helm and Wedemann,[5] the addition of lime to sodium-hydroxide solutions increases their efficiency against the virus of foot-and-mouth disease. On the other hand, the work of Petroff [6] and of Corper and

[4] OLITSKY, P. K., TRAUM, J., and SCHOENING, H. W. REPORT OF THE FOOT-AND-MOUTH-DISEASE COMMISSION OF THE UNITED STATES DEPARTMENT OF AGRICULTURE. U. S. Dept. Agr. Tech. Bul. 76, 172 p., illus. 1928.

[5] HELM, R., and WEDEMANN, W. VERSUCHE MIT VERSCHIEDENEN DISINFEKTIONSMITTELN ZUR ABTÖTUNG DES VIRUS DER MAUL-UND-KLAUENSEUCHE. Arb. Reichsgsndhtsamt. [Germany] 61: 569–594. 1930.

[6] PETROFF, S. A. A NEW AND RAPID METHOD FOR THE ISOLATION AND CULTIVATION OF TUBERCLE BACILLI DIRECTLY FROM SPUTUM AND FECES. Jour. Expt. Med. 21: 38–42. 1915.

Uyei [7] indicates that sodium hydroxide has practically no germicidal efficiency against the tubercle bacillus. During the course of the present work the writers tested mixtures containing 2 per cent of sodium hydroxide and 10 per cent of lime against the tubercle bacillus and found them to be ineffective after two hours' exposure.

For purposes of general disinfection it is suggested that sodium hydroxide should be used in 2 per cent solution with or without lime. When circumstances permit its use, the solution containing lime will no doubt be preferred, since the lime not only serves to indicate where the solution has been applied but also increases germicidal efficiency, and tends to maintain the strength of the sodium hydroxide. When spore-bearing organisms are to be destroyed, it is suggested that sodium hydroxide, with or without lime, should be used with initial concentrations of from 5 to 10 per cent.

Sodium hydroxide is available commercially as caustic soda or lye in a very pure state. Since caustic soda which assays more than 90 per cent sodium hydroxide is readily obtainable, it would seem that a good grade of the commercial product might be used for practical disinfecting operations.

Although caustic soda itself and concentrated solutions of it are corrosive, this injurious effect is greatly diminished by dilution. The dilute solutions here suggested for general disinfection may be used with safety, provided a reasonable degree of care is exercised. Such solutions have little or no harmful effect upon rubber, bare wood, or cotton; but repeated treatment is harmful to wool or silk. Leather, especially when oiled, and many kinds of painted surfaces withstand some exposure to these solutions, but it is better to avoid unnecessary exposure. The solutions may be kept in vessels of wood, earthenware, enameled ware, or any of the common metals with the exception of aluminum. Containers should be tightly covered to prevent conversion of hydroxide to carbonate by exposure to air. In handling the stronger solutions suggested for use against spore-bearing organisms, special care should be exercised.

For cleansing and disinfecting it is suggested that sodium carbonate be used in a 2 per cent solution. This may be obtained by using commercial soda at the rate of 6 parts of soda crystals, or 2 parts of soda ash, in 100 parts of water; or trisodium phosphate may be used in a 1 per cent solution, which may be obtained by using the commercial tribasic phosphate of soda in the proportion of 2.5 parts in 100 parts of water. If these solutions are to be used at ordinary temperatures, it is advisable to add 0.5 per cent of sodium hydroxide. If the solutions are to be used hot, the addition of sodium hydroxide will not be necessary.

SUMMARY

Bacteriological tests were made with sodium hydroxide, sodium carbonate, trisodium phosphate, mixtures containing sodium carbonate or trisodium phosphate with sodium hydroxide or with soaps, and mixtures containing sodium hydroxide and calcium hydroxide. The test organisms used were *Eberthella typhi*, *Staphylococcus aureus*, *Salmonella pullorum*, *Salmonella gallinarum*, *Pasteurella avicida*, two strains of hemolytic streptococci, and spores of *Bacillus anthracis*.

[7] CORPER, H. J., and UYEI, N. THE ISOLATION OF TUBERCLE BACILLI FROM CONTAMINATED TUBERCULOUS MATERIALS. Tubercle 9: 115-130. 1927.

Sodium hydroxide, alone or in mixtures with sodium carbonate, trisodium phosphate, or calcium hydroxide, was found to have relatively high germicidal efficiency against all these organisms, even in the presence of organic matter in the form of skim milk, chicken feces, or defibrinated horse blood. During the course of the work it was found that solutions containing 2 per cent sodium hydroxide and 10 per cent calcium hydroxide failed to kill *Mycobacterium tuberculosis* without organic matter after two hours' exposure.

Sodium carbonate, trisodium phosphate, and calcium hydroxide each had comparatively little germicidal efficiency at ordinary temperatures, but when added to solutions of sodium hydroxide they increased the germicidal efficiency of these solutions. The germicidal efficiency of solutions of sodium carbonate or trisodium phosphate or of mixtures containing either of these compounds with sodium hydroxide was greatly increased by heat. The addition of sodium carbonate or trisodium phosphate to soap solutions increased their germicidal efficiency.

A METHOD FOR THE PREPARATION AND ANALYSIS OF REPRESENTATIVE SAMPLES FROM THE BOVINE SKELETAL STRUCTURE[1]

By W. M. NEAL,[2] *Research Assistant*, and L. S. PALMER, *Dairy Chemist, Division of Agricultural Biochemistry, Minnesota Agricultural Experiment Station*

INTRODUCTION

In the past, studies of the composition of the skeletal structure of animals have been limited to rather gross analyses; moisture, fat, and ash being the constituents usually determined. Recently, however, methods have been devised for more detailed studies, so that the constituents of the mineral matter can also be determined. Information so gained is necessary for an understanding of the complete effects of various deficiencies in nutrition.

When animals that had been used in mineral nutrition projects at the Minnesota station were slaughtered an opportunity was afforded to study the effect of age and nutrition on the skeletal structure of cattle. The old method of grinding an entire half skeleton requires a large amount of labor. Other methods lack experimental support. The method to be described was developed to avoid this labor, and yet to furnish a sample that would adequately represent the skeleton. Preliminary studies indicated that comparative results could be secured, and the data obtained support this conclusion.

The use of the femur, humerus, and ribs in small-animal experimentation prompted their selection to represent the entire skeleton of cattle in this study. These bones represent both endochondral and intramembranous ossification, and hence should be affected by changes in nutrition proportionately with the other bones. However, there is as yet no direct evidence on this point.

A considerable amount of labor is required to reduce a whole femur or humerus from a large cow into material suitable for analysis. In order to eliminate as much of this labor as possible and at the same time to reduce the size of the sample, various fractions of these bones were analyzed, and the results of the analyses were compared with those for the whole bone. It was concluded from this work that a longitudinal section could be sawed from either the femur or the humerus which would closely approximate the whole bone in composition. The method employed will first be described, and then the data in support of it.

THE METHOD

The femur, humerus, sixth and eleventh ribs from the right side of the bovine carcass were removed as soon as possible after the animal was slaughtered. These bones with the adhering flesh were

[1] Received for publication Aug. 28, 1930; issued January, 1931. This, the first of two articles relating to the influence of age and nutrition on the major mineral constituents of the skeletal structure of cattle, was presented by the senior author to the faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of doctor of philosophy, June, 1929. Paper No. 949, Journal Series, Minnesota Agricultural Experiment Station.

[2] Resigned June 30, 1929.

frozen immediately. When this was done the flesh was scraped from the bone and longitudinal sections of the femur and humerus were sawed. These sections and the ribs were then broken in an iron mortar and weighed into tared aluminum dishes. The type of saw used and the character of the bone samples are shown in Figure 1. The longitudinal section shown is of uniform thickness when prepared with the modified hack saw. It is obtained most easily from the frozen bone. This section more nearly represents proportionately the

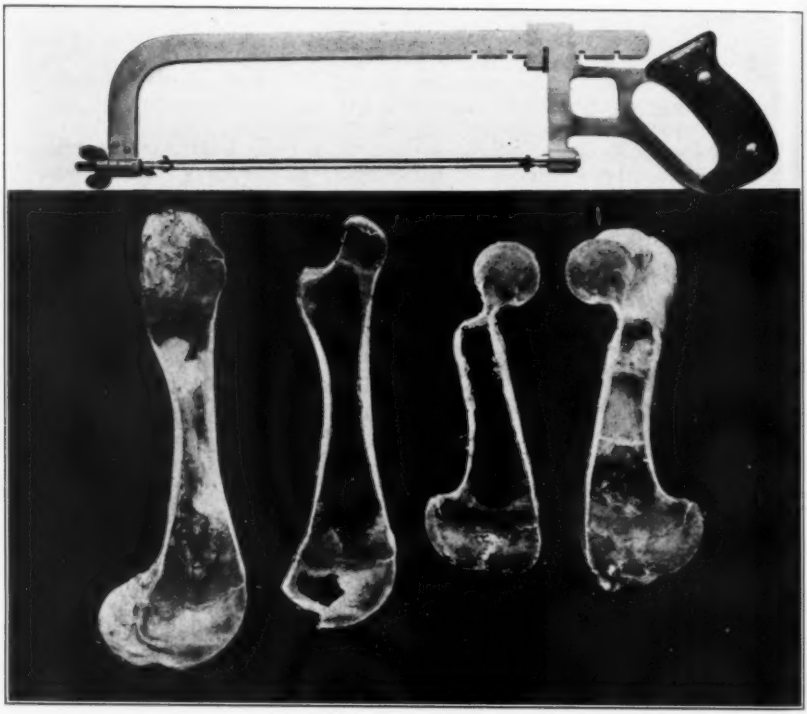

FIGURE 1.—Double-bitted hack saw used to cut longitudinal sections of bone for samples as shown

different parts of the bone than the cross section usually made. No other equally representative sample of a large bone can be secured so easily or prepared for analysis with less labor.

All samples were dried to constant weight at 100° C. When dried they were transferred to extraction thimbles, placed in a Lloyd drug extractor, and extracted with hot 95 per cent alcohol followed by ether until they were extract-free. The percentage of alcohol-ether extract was calculated from the difference between the dry and dry-extracted weights. The sample was then reduced in an iron mortar until it would pass a 20-mesh sieve. Large samples were ground in a Hobart mill. Ash determinations were made on gram aliquots of this bone powder.

The mineral constituents of the dry extracted bone were determined by the method of Kramer and Howland [3] for unashed bone, except for two modifications. One modification was the precipitation, washing, and titration of the calcium in 75 c. c. pointed centrifuge tubes as for blood calcium,[4] but using the quantities of sample and reagents recommended by Kramer and Howland. The other was the determination of the carbonate by absorption in standard alkali, using a set-up very similar to that employed in the Geissler method. (Fig. 2.)

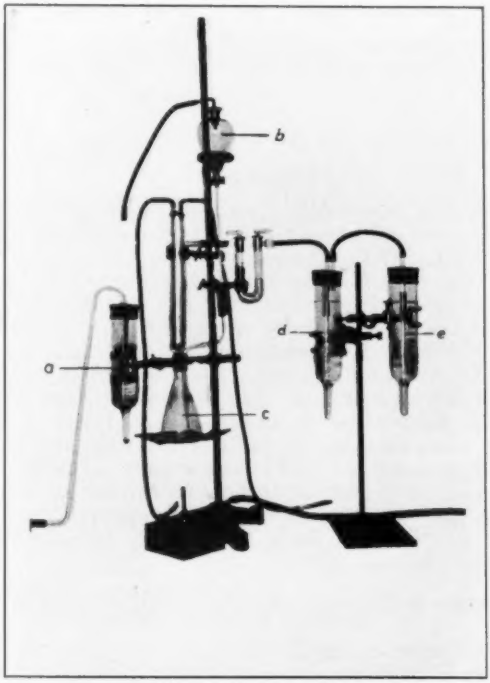

FIGURE 2.—Apparatus for the determination of carbonate by CO_2 absorption in standard alkali: *a*, Absorption tube containing 40 per cent KOH solution; *b*, separatory funnel for adding HCl for liberation of CO_2; *c*, flask containing sample of bone powder; *d–e*, tubes containing standard alkali for absorption of CO_2

A 1.25 gm. sample of bone powder was weighed into the flask *c*, and 25 c. c. of 1 N hydrochloric acid was added through the separatory funnel *b* for the liberation of the carbon dioxide. Carbon dioxide-free air was furnished by aspiration through the absorption tube *a* containing 40 per cent potassium hydroxide solution. Aspiration and heating were continued for 30 minutes at the rate of about two bubbles per second. The reflux condenser and the U-tube con-

[3] KRAMER, B., and HOWLAND, J. THE QUANTITATIVE ESTIMATION OF CALCIUM, MAGNESIUM, PHOSPHATE, AND CARBONATE IN BONE. Jour. Biol. Chem. 68: 711–719. 1926.

[4] KRAMER, B., and TISDALL, F. F. A SIMPLE TECHNIQUE FOR THE DETERMINATION OF CALCIUM AND MANGESIUM IN SMALL AMOUNTS OF SERUM. Jour. Biol. Chem. 47: 475–481. 1921.

taining sulphuric acid (specific gravity 1.4) saturated with silver sulphate prevented any hydrochloric acid from contaminating the standard alkali in the tubes *d* and *e*. A 50 c. c. portion of alkali (1 c. c. equal 1 mgm. CO_2) was used in each of these tubes.

After the absorption of the carbon dioxide was completed, 5 c. c. of 10 per cent barium chloride was added to each tube and the excess alkali titrated with standard acid, phenolphthalein being used as the indicator.

After the carbonate determination the sample was filtered into a volumetric flask, and aliquots were taken for the calcium, magnesium, and phosphorus determinations. The aliquots taken were equivalent in amount to those used in the Kramer and Howland [5] method. From the data obtained the ratios of residual calcium to residual phosphorus and of calcium phosphate to calcium carbonate were calculated according to the Kramer and Howland method.

EXPERIMENTAL DATA

The analyses of the various fractions of a femur and of two longitudinal sections of a humerus as well as the whole bones of E-73 are given in Table 1. With the exception of fraction 7 none of the other fractions of the femur compared so closely in all respects with the whole bone as did the longitudinal section. Fraction 7 was not chosen because of the obvious difficulty of duplicating it in other bones and also because its mechanical reduction for analysis is just as difficult as in the case of the entire bone. The two sections of the humerus agree sufficiently well to show that a uniform section may be taken. That the percentage of ash in the dry extracted section is higher than that for the whole bone is due, probably, to a slightly higher proportion of the shaft being included in the section. The section is of uniform thickness, while the whole bone is thicker at the extremities. However, it may be supposed that this disproportionality would occur in all samples thus taken, so that the samples would still be comparable one with another.

TABLE 1.—*Comparison of percentage composition of various fractions* [a] *of femur and humerus, and whole bone*

Basis of calculation	Moisture (green) bone	Alcohol ether extract		Ash		
		Green bone	Dry bone	Green bone	Dry bone	Extract bone
Femur E-73:	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Longitudinal section	12.23	34.53	39.34	35.24	40.15	66.19
Fraction 1	19.08	36.37	44.95	27.26	53.68	61.19
Fraction 2	13.72	26.47	30.68	38.01	44.07	63.57
Fraction 3	13.39	33.47	38.65	32.19	37.17	60.59
Fraction 4	13.43	21.50	24.83	42.17	48.71	64.80
Fraction 5	12.18	35.01	39.86	34.56	39.29	65.33
Fraction 6	12.17	19.65	22.36	45.02	51.26	66.03
Fraction 7	12.83	30.85	35.16	36.64	42.03	64.83
Fraction 8	12.29	35.44	40.41	34.26	39.06	65.55
Whole bone	14.04	31.23	36.33	34.88	40.58	63.73
Humerus E-73:						
First longitudinal section	10.80	32.02	35.91	37.00	41.49	64.73
Second longitudinal section	11.65	31.45	35.59	36.81	41.67	64.70
Whole bone	11.09	35.67	37.87	34.76	39.06	62.84

[a] After the section was sawed, the half bone was divided again both longitudinally and crosswise through the shaft, each half yielding four fractions.

[5] KRAMER, B., and HOWLAND, J. Op. cit.

Since the analyses of these sections of the femur and humerus of E-73 gave results closely comparable to those for the whole bones, the work was continued, and the method outlined above was used on all available samples. Most attention was paid to the calcium phosphate/calcium carbonate ratio, as one of the objectives of the research was to determine the effect of different planes of calcium and phosphorus intake on the composition of the mineral matter in the skeleton.

The calcium phosphate/calcium carbonate ratios for the femur, humerus, and combined sample of the sixth and eleventh ribs for 16 animals considered normal, are given in Table 2.

TABLE 2.—*Calcium phosphate/calcium carbonate ratios for the femur, humerus, and sixth and eleventh ribs of 16 normal animals*

Animal No.	Age	Calcium phosphate/calcium carbonate ratio in—			
		Femur	Humerus	Ribs	Average ratio
	Months				
E-106	6	7.53	7.58	7.11	7.41
E-114	6	7.34	6.88	7.09	7.10
B-163	6	6.57	7.46	7.25	7.09
E-79	12	7.38	7.04	7.39	7.27
E-55	18	6.47	7.12	7.17	6.92
E-85	18	6.99	7.30	7.14	7.14
E-88	18	7.35	6.94	7.32	7.20
E-86	21	7.54	7.33	7.24	7.37
(a)	(?)	7.87	7.72	7.97	7.85
E-78	24	7.38	7.46	7.58	7.47
E-98	24	6.75	6.70	6.52	6.66
E-23	(b)	6.91	6.69	6.68	6.76
310	(b)	6.70	6.07	6.73	6.50
101	(b)	6.88	6.47	6.42	6.59
19	(b)	6.56	6.40	6.28	6.41
E-62	(b)	6.44	6.96	6.91	6.77

[a] Jersey bull. [b] Mature.

Correlations were calculated with the following results:

$$r \text{ femur-humerus} = +0.53136 \pm 0.11736 \quad (N=16)$$
$$r \text{ femur-ribs} = +0.66739 \pm 0.09352 \quad (N=16)$$
$$r \text{ humerus-ribs} = +0.81416 \pm 0.05685 \quad (N=16)$$
$$r \text{ average-ribs} = +0.83353 \pm 0.05454 \quad (N=16)$$

These correlations show that the calcium phosphate/calcium carbonate ratio of the humerus samples agrees very closely with that of the ribs, and that the ratio for the ribs is very close to the average ratio for the three samples. The ribs may thus be considered as the most reliable single sample.

It can not be concluded from these correlations, however, that the samples from the ribs give as accurate values as the means of the three samples. This is due to the unreliability of the last correlation coefficient caused by one variable being independent and the other dependent. Harris [6] has pointed out that a more significant value, the rate of variation of the dependent variable in relation to the independent variable, can be secured by a correlation of the independent variable with the deviation of the dependent variable from its most probable value. Applying Harris's [7] formula with the value

[6] HARRIS, J. A. THE CORRELATION BETWEEN A VARIABLE AND THE DEVIATION OF A DEPENDENT VARIABLE FROM ITS PROBABLE VALUE. Biometrika 6: 438–443. 1909.

[7] HARRIS, J. A. Op. cit.

for the ribs as the dependent variable, the value -0.13913 ± 0.16536 was obtained. This indicates that the ribs are a representative sample because the ratio for the ribs varies uniformly with the average for the three samples.

The availability of some cross sections of shaft made it desirable to determine whether the ratio obtained from the analysis of such a section would be comparable to that obtained from a longitudinal section as was adopted for this study. Table 3 gives a comparison of longitudinal and cross sections from the femurs and humeri of four animals.

TABLE 3.—*Comparison of calcium phosphate/calcium carbonate and calcium/phosphorus ratios of longitudianal and cross sections of the same bone of animals on normal and low calcium rations*

Animal No.	Age	Bone	Ratios in—			
			Longitudinal sections		Cross section	
			$\frac{Ca_3(PO_4)_2}{CaCO_3}$	$\frac{^a Ca}{P}$	$\frac{Ca_3(PO_4)_2}{CaCO_3}$	$\frac{^a Ca}{P}$
	Months					
E-106	6	Femur	7.53	1.88	6.74	1.87
		Humerus	7.58	1.87	6.71	1.87
E-114	6	Femur	7.34	1.90	6.90	1.89
		Humerus	6.88	1.90	6.69	1.85
ANIMALS ON A LOW CALCIUM RATION						
E-109	12	Femur	6.95	1.92	7.00	1.86
		Humerus	6.89	1.87	6.57	1.87
E-110	12	Femur	7.41	1.90	7.08	1.88
		Humerus	7.11	1.90	7.04	1.88

[a] Residual.

The $\frac{Ca_3(PO_4)_2}{CaCO_3}$ ratios for the samples from the animals on the low calcium rations agree fairly well. There is no such agreement for those from the animals on normal rations. The peculiarity of the low calcium ration apparently was to produce a mineral of more nearly the same composition as that already present in the shaft. Shear and Kramer [8] found differences in the composition of new and old calcification, and similar differences may account for the variations observed here. The necessity for a sample including all parts of the bone is evident.

When the analyses were completed, correlation coefficients were calculated as shown in Table 4. All animals from which the three samples had been taken are included. Fifteen animals had been on normal rations and 12 on abnormal mineral rations. These latter 12 are included in the total of 27. The lowest coefficient, $+0.3919 \pm 0.1474$, was between the magnesium values for the humeri and ribs. It is a little less than three times the probable error. Almost all the coefficients are greater than $+0.7$ and the highest, $+0.934933 \pm 0.016343$, is unusually high for biological material.

[8] SHEAR, M. J., and KRAMER, B. COMPOSITION OF BONE.—III. PHYSICOCHEMICAL MECHANISM. Jour. Biol. Chem. 79: 125–145. 1928.

On the assumption that there should be a high correlation between the quantity of any constituent in the various parts of the skeleton, these correlation coefficients indicate success in the selection of representative samples. The conclusion seems justified that longitudinal sections of the femur and humerus, and the sixth and eleventh ribs do adequately represent the bovine skeletal structure.

SUMMARY

A method for the preparation and analysis of samples from the bovine skeleton has been described.

Data are presented to show that longitudinal sections of the femur and humerus and the sixth and eleventh ribs constitute representative samples of the bovine skeletal structure.

TABLE 4.—*Correlation coefficients calculated between the several analyses*[a]

Number of animals considered	r F-R [b]	r H-R [b]	r FH-R [b]	r HR-F [b]	r FR-H [b]
CALCIUM					
15	+0.6193±0.1093	+0.7332±0.0805			
27	+.7686±.0700	+.7143±.0636	+0.7267±0.0612	+0.8412±0.0422	+0.8490±0.0362
MAGNESIUM					
15	+.5059±.1296	+.3919±.1476			
27	+.7906±.0487	+.7361±.0595	+.7817±.0505	+.9082±.0227	+.8592±.0340
PHOSPHORUS					
15	+.5715±.1173	+.7284±.0817			
27	+.7210±.0623	+.6314±.0780	+.7217±.0622	+.8344±.0304	+.7740±.0520
CARBON DIOXIDE					
15	+.7416±.0784	+.7517±.0757			
27	+.8799±.0293	+.8866±.0278	+.9039±.0237	+.9228±.0193	+.9294±.0187
CALCIUM PHOSPHATE/CALCIUM CARBONATE					
15	+.6853±.0924	+.8150±.0584	+.8475±.0491	+.6634±.0975	+.7619±.0730
27	+.8548±.0349	+.9237±.0190	+.9349±.0163	+.8691±.0362	+.9012±.0243
15	r FHR-R +0.9352±0.0218				

[a] Credit is due Rachel Rude, station assistant, for the calculation of the correlation coefficients presented in this table.

[b] F=femur, H=humerus, R=ribs, FH=average of femur and humerus, etc.

EFFECT OF AGE AND NUTRITION ON THE CALCIUM PHOSPHATE/CALCIUM CARBONATE RATION IN THE BONES OF CATTLE[1]

By W. M. NEAL,[2] *Research Assistant*, and L. S. PALMER, *Dairy Chemist, Division of Agricultural Biochemistry*, and C. H. ECKLES *Chief*, and T. W. GULLICKSON, *Assistant Dairy Husbandman, Division of Dairy Husbandry, Minnesota Agricultural Experiment Station*

INTRODUCTION

A knowledge of the changes induced in the skeletal structure of cattle by different planes of mineral nutrition should aid in determining the optimum mineral intake for the development of the skeleton and its maintenance in normal condition. The proportions as well as the total quantity of the mineral constituents may be altered through nutrition. Only in recent years have methods been devised for the study of these changes. Kramer and Howland (*7*) [3] provided such a method, and its adaptation for the analysis of the skeletons of cattle has been described in a previous paper (*11*).

This method of analysis permits the determination of calcium, magnesium, phosphorus, and carbonate on unashed bone. Since these comprise the constituents of the mineral matter, except for minute amounts of some other elements, it is possible to make deductions concerning some of the most probable salts and their proportions. By assuming that the carbonate is all present as calcium carbonate and the magnesium as trimagnesium phosphate, it was found that the remaining calcium and phosphorus were generally in nearly the same proportion as for tricalcium phosphate. Using this result in support of the assumption that the calcium phosphate is tricalcium phosphate, the ratio of tricalcium phosphate to calcium carbonate may be calculated.

In spite of the fact that only small variations may be secured in the Ca/P ration of bone ash (*4*, *5*, *9*, *10*, *12*), Howland, Marriott, and Kramer (*6*) and Kramer and Shear (*8*) found significant variations in the composition of calcification by analyzing unashed samples of bone. They reported the calcium phosphate/calcium carbonate ratio for the bones of normal rats to be 10.8 and 10.6, and for a rachitic rat 6.7. A similar reduction of the ratio was observed in rachitic human bones. A constant ratio would not be expected. The concentration of the ions in the body fluids surrounding the ossifying tissue is not constant and this should be reflected in the resulting ossification.

[1] Received for publication Aug. 28, 1930; issued January, 1931. This, the second of two articles relating to the influence of age and nutrition on the major mineral constituents of the skeletal structure of cattle, was presented by the first-named author to the faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of doctor of philosophy, June, 1929. Paper No. 950, Journal Series, Minnesota Agricultural Experiment Station.

[2] Resigned June 30, 1929.

[3] Reference is made by number (italic) to Literature Cited, p. 121.

EXPERIMENTAL DATA

So far as the writers are aware, no analyses of unashed bones of cattle have ever been reported. It was believed that such a study performed on the skeletons of cattle reared or maintained on rations of known calcium and phosphorus content would aid in the elucidation of the requirement for these elements. Normal mature cattle were taken from the University Farm herd where there has never been any evidence of mineral deficiency. Mature cattle that had received a phosphorus-deficient ration were available from the experiments of Eckles, Becker, and Palmer (*3*). Other animals used in the same project had received a phosphorus-deficient ration which was later supplemented with sodium or calcium phosphate. The younger animals were all from a project on the mineral requirements for growth. These latter animals were grouped according to the amount of calcium and phosphorus received.

Whether the supply of calcium and phosphorus was considered as low, medium, or high, was based on the computation made by Armsby (*1*) from the analyses of Lawes and Gilbert showing that the average daily storage is 3.2 gm. of calcium and 1.7 gm. of phosphorus per hundred pounds live weight. An animal receiving a moderate excess over these amounts was considered to be receiving a normal mineral ration. An average ration of mixed hay and grain supplies a slight excess of calcium and a liberal amount of phosphorus. Lack of data on the percentage assimilation of calcium and phosphorus by growing cattle precludes a definite statement as to the absolute adequacy of such a ration.

Natural feeds were used in all the rations and were assumed to supply sufficient vitamin D. Regardless of the quantitative intake of this vitamin, it was sufficiently uniform to prevent its being the determining factor in the composition of the mineral of the skeletal structure.

The ratio of residual calcium to residual phosphorus is not shown in this paper because in all cases it closely approximated the value for tricalcium phosphate, and so was not of additional significance.

Twenty animals were included in the normal group. The composition of the skeletons of the younger animals varied. Lack of absolute uniformity in the rations, individuality of the animals, and differences in their early history are probable causes of this variation. The average amount of calcium and phosphorus supplied was more than enough to care for the usual storage. All animals were normal in size and appearance.

Average ratios of the calcium phosphate to calcium carbonate obtained, as shown in Table 1, were: 7.32 for those slaughtered at 6, 9, and 12 months, 7.11 for those at 18 and 24 months, and 6.57 for mature cows. The decrease in the second year is very slight, but its occurrence is substantiated by the further decrease in mature cows—probably due to a more uniform mineral intake. These results for normal cattle serve as a standard of comparison for the cattle on abnormal mineral rations.

The cows on a known phosphorus-deficient ration received prairie hay grown in the phosphorus-deficient area in western Minnesota, ground oats, common salt, and water. The ratios for five such animals are presented in Table 2, the average for the low four being

5.30. E–37 was omitted from this average because she was not in milk, and no longer showed symptoms of mineral deficiency at the time of slaughter. A very liberal proportion of ground oats in her ration apparently had supplied her phosphorus requirement.

TABLE 1.—*Effect of age on the calcium phosphate/calcium carbonate ratio in the skeletons of dairy cattle on normal rations*

Animal No.	Age	$\frac{Ca_3(PO_4)_2}{CaCO_3}$	Standard deviation
	Months		
E–106	6	7.41	
E–114	6	7.10	
B–163	6	7.09	
E–102	9	7.73	
E–79	12	7.27	
Average		7.32	±0.237
E–55	18	6.92	
E–85	18	7.14	
E–88	18	7.20	
WVa–3 [a]	18	6.78	
WVa–6	18	7.23	
E–86	24	7.37	
E–78	24	7.47	
E–98	24	6.66	
WVa–4	24	7.02	
WVa–5	24	7.30	
Average		7.11	± .236
E–25	[b]	6.59	
E–23	[b]	6.76	
310	[b]	6.50	
101	[b]	6.59	
19	[b]	6.41	
Average		6.57	± .116

[a] Samples of the bones of cattle numbered with the prefix WVa were furnished by H. O. Henderson of the West Virginia Agricultural Experiment Station.
[b] Mature.

TABLE 2.—*Calcium phospate/calcium carbonate ratio in the skeletons of mature dairy cattle fed rations containing different levels of phosphorus*

ANIMALS ON A PHOSPHORUS-DEFICIENT RATION

Animal name or No.	$\frac{Ca_3(PO_4)_2}{CaCO_3}$	Standard deviation
Daisy	4.84	
E–36	5.36	
E–37	[a] 6.22	
E–73	5.66	
E–90	5.34	
Average	5.30	±0.294

ANIMALS ON A PHOSPHORUS-DEFICIENT RATION SUPPLEMENTED WITH SODIUM OR CALCIUM PHOSPHATE

E–34	6.30	
E–58	6.31	
E–59	6.20	
E–61	6.29	
E–62	6.77	
Average	6.37	±0.202

[a] Omitted from the average.

The animal called Daisy showed symptoms of extreme phosphorus deficiency. When she was slaughtered her ribs were found to be extremely soft and were readily pierced with a knife. E–90 was very emaciated, showed depraved appetite, and her joints creaked when she walked. The inorganic phosphorus in her plasma as determined by the Briggs (*2*) method was nearly 1.00 mg. per 100 c. c. of plasma. E–36 was in a similar condition. E–73 did not show such acute symptoms of phosphorus deficiency, but would chew bones. The inorganic phosphorus in her plasma averaged 2.00 mg. per 100 c. c. of plasma for the six months previous to slaughter.

Daisy, E–90, and E–36 showed the lowest ratios, E–73 somewhat higher, and E–37 a nearly normal ratio. This was in direct agreement with the degree of deficiency exhibited. The average ratio, 5.30, is almost the same as that reported for rachitic rats *(6)*. The relative decrease in the amount of calcium phosphate indicates that there is a selective absorption during periods of deficiency. The animal in drawing on its mineral reserve in the skeleton for phosphorus either did not withdraw a proportionate amount of carbonate, or even replaced some of the calcium phosphate with calcium carbonate. The result may also indicate that the metabolism of the bone carbonates is independent of that of the phosphates.

It has been shown (*3*) that the feeding of the phosphorus-deficient ration supplemented with either sodium or calcium phosphate will result in normal reproduction, increased lactation, and gain in body weight. The bones of five animals so fed were analyzed. Before the feeding of the supplement the condition of these animals was comparable to that of the phosphorus-deficient group. E–62, which received the supplement longest, showed the highest ratio, 6.77. (Table 2.) All the ratios were within the range of the normal, and from this it is concluded that the skeletal structures of the animals had recovered their normal proportion of calcium phosphate and calcium carbonate. These are believed to be the first analyses reported from cattle after a period of phosphorus-deficiency followed in turn by adequate rations, and they indicate that recovery from such a condition can be complete.

Less is known of the effect of phosphorus deficiency on growing cattle than on mature ones. Henderson and Weakley (*5*) found an increase in the moisture content and a decrease in the breaking strength of bones from cattle raised on a low phosphorus ration. Bone samples from eight of their animals on low phosphorus rations are included in this study. (Table 3.) During the first year these animals received an ample quantity of phosphorus, but during the second year they were provided with only 60 to 75 per cent of the phosphorus usually retained.

TABLE 3.—*Calcium phosphate/calcium carbonate ratio in the skeletons of young dairy cattle fed rations containing different levels of phosphorus* [a]

Animal No.	Age	$Ca_3(PO_4)_2$ / $CaCO_3$	Standard deviation
ANIMALS ON A LOW PHOSPHORUS RATION			
	Months		
WVa-27	18	5.94	
WVa-30	18	6.12	
WVa-19	18	6.49	
WVa-22	18	6.11	
Average		6.16	±0.201
WVa-28	24	6.86	
WVa-29	24	6.39	
WVa-20	24	6.92	
WVa-21	24	6.51	
Average	24	6.67	±.225
ANIMALS ON A HIGH PHOSPHORUS RATION			
E-87	6	8.04	
E-96	6	7.65	
Average		7.85	

[a] The bone samples from animals on a low phosphorus ration were furnished by Doctor Henderson and Professor Weakley of the West Virginia Agricultural Experiment Station and represented samples used by them in another study (*5*). We acknowledge their kindness in supplying us with this material.

The calcium phosphate/calcium carbonate ratio was reduced in these animals. The normal ratios for cattle 18 and 24 months old were 7.05 and 7.16, respectively, as compared with 6.16 and 6.67 for these animals. Henderson observed some improvement in their condition during the last six months, and attributed it to a lessened phosphorus requirement which made a partial recovery possible. The ratios observed support this explanation of their improvement.

One-half of the animals of this group received a ration that was low in both calcium and phosphorus. There was no apparent ill effect from the low calcium, possibly because the deficiency of phorphorus reduced the requirement for calcium by preventing the usual amount of new calcification. The rations used were not so low in phosphorus as those employed at University Farm, nor were the ratios obtained so low. However, considering the difference in the normal ratios for the two ages, comparable reductions were secured in both cases. The higher mineral requirement of the young animal for skeletal growth makes it possible to secure a deficiency on rations carrying appreciable quantities of phosphorus.

It was planned to study the effect of low, medium, and high calcium and phosphorus intake on the growth and development of dairy cattle. Only two animals were available in the high phosphorus group, and none in the medium group when these data were obtained. (Table 3.) Both of the animals in the high phosphorus group were slaughtered at 6 months of age, and the high ratio of 7.85 was secured from the analyses of the bone samples. This raises the question whether an abnormally high reserve of phosphorus can be stored in the skeleton, and if so, would it not aid in meeting the phosphorus requirement during later lactation? It seems improbable that there should be any deleterious effects from high phosphorus intake in

view of the quantity of supplement (100 gm. of sodium or calcium phosphate daily) fed to cows on phosphorus-deficient rations (*3*).

The necessity of feeding milk to calves made it impossible to secure satisfactory low calcium rations for the first six months. Although the usual calcium intake was close to the amount generally retained, some departure from the normal was observed in three of the calves slaughtered at 6 or 12 months. (Table 4.) Three of those slaughtered at 18 and 24 months showed an increase in the calcium phosphate/calcium carbonate ratio, while the fourth was practically normal. However, there were too few animals and the calcium intake was not sufficiently low to warrant any definite conclusions. Indications are that either an increase or a decrease in the proportion of phosphate in the skeleton may occur as a result of calcium deficiency.

TABLE 4.—*Calcium phosphate/calcium carbonate ratio in the skeletons of young dairy cattle fed rations containing different levels of calcium*

Animal No.	Age	$\frac{Ca_3(PO_4)_2}{CaCO_3}$	Standard deviation
ANIMALS ON A LOW CALCIUM RATION			
	Months		
E-103	6	6.60	
E-104	6	6.91	
E-109	12	6.85	
E-110	12	7.23	
Average		6.90	±0.224
WVa-11	18	7.75	
WVa-14	18	7.76	
WVa-12	24	7.61	
WVa-13	24	7.27	
Average		7.60	±.198
ANIMALS ON A HIGH CALCIUM RATION			
E-95	12	7.58	
E-89	12	7.25	
Average		7.41	

There were but two animals on a high calcium ration. The ratio of calcium phosphate to calcium carbonate was normal when they were slaughtered at 12 months of age.

CONCLUSION

The residual calcium/residual phosphorus ratio of cattle bone approximates the calcium/phosphorus ratio of tricalcium phosphate. The residual calcium/residual phosphorus ratio of the bone is not affected by calcium or phosphorus deficiency in the ration. This is the ratio between the calcium and phosphorus which do not occur in the bone as calcium carbonate or trimagnesium phosphate; it always closely approximates the value for tricalcium phosphate.

The calcium phosphate/calcium carbonate ratio of the bones of dairy cattle is affected by age and nutrition. This ratio decreases with age. Phosphorus deficiency causes a decreased ratio, but feed-

ing a phosphorus supplement permits the recovery of a normal ratio. Rations high in phosphorus may produce a high ratio.

There were indications that either an increase or a decrease in the proportion of phosphate in the skeleton may occur as a result of calcium deficiency, although the results did not warrant definite conclusions. The ratio of calcium phosphate to calcium carbonate was normal in two animals on a high calcium ration when slaughtered at 12 months of age.

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